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<p>(54) Title: NOVEL PESTICIDAL PROTEINS AND STRAINS</p> <p>(57) Abstract</p> <p>The present invention is drawn to pesticidal strains and proteins. <i>Bacillus</i> strains which are capable of producing pesticidal proteins and auxiliary proteins during vegetative growth are provided. Also provided are the purified proteins, nucleotide sequences encoding the proteins and methods for using the strains, proteins and genes for controlling pests.</p>		

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## **NOVEL PESTICIDAL PROTEINS AND STRAINS**

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The present invention is a continuation-in-part application of U.S. application serial number 08/037,057 filed March 25, 1993, the disclosures of which are herein incorporated by reference.

10

### **FIELD OF THE INVENTION**

The present invention is drawn to methods and compositions for controlling plant and non-plant pests.

15

### **BACKGROUND OF THE INVENTION**

20

Insect pests are a major factor in the loss of the world's commercially important agricultural crops. Broad spectrum chemical pesticides have been used extensively to control or eradicate pests of agricultural importance. There is, however, substantial interest in developing effective alternative pesticides.

25

Microbial pesticides have played an important role as alternatives to chemical pest control. The most extensively used microbial product is based on the bacterium Bacillus thuringiensis (Bt). Bt is a gram-positive spore forming Bacillus which produces an insecticidal crystal protein (ICP) during sporulation.

Numerous varieties of Bt are known that produce more than 25 different but related ICP's. The ICP's made by Bt are toxic to larvae of certain insects in the orders Lepidoptera,

Diptera and Coleoptera. In general, when the ICP is ingested by a susceptible insect the crystal is solubilized and transformed into a toxic moiety by the insect gut proteases. None of the ICP's active against coleopteran larvae have demonstrated significant effects on the genus Diabrotica particularly Diabrotica virgifera virgifera, the western corn rootworm (WCRW) or Diabrotica longicornis barberi, the northern corn rootworm.

Bt is closely related to Bacillus cereus (Bc). A major distinguishing characteristic is the lack of a parasporal crystal in Bc. Bc is a widely distributed bacterium that is commonly found in soil and has been isolated from a variety of foods and drugs. The organism has been implicated in the spoilage of food.

Although Bt has been very useful in controlling insect pests, there is a need to expand the number of potential biological control agents.

#### SUMMARY OF THE INVENTION

The present invention is drawn to compositions and methods for controlling plant and non-plant pests. Particularly, new pesticidal proteins are disclosed which are isolatable from the vegetative growth stage of Bacillus. Bacillus strains, proteins, and genes encoding the proteins are provided.

The methods and compositions of the invention may be used in a variety of systems for controlling plant and non-plant pests.

### DETAILED DESCRIPTION OF THE INVENTION

Compositions and methods for controlling plant pests are provided. In particular, novel pesticidal proteins are provided which are produced during vegetative growth of Bacillus strains.

5 The proteins are useful as pesticidal agents.

The present invention recognizes that pesticidal proteins are produced during vegetative growth of Bacillus strains. For the purpose of the present invention vegetative growth is defined as that period of time before the onset of sporulation. In the case of Bt, this vegetative growth occurs before production of ICPs. Genes encoding such proteins can be isolated, cloned and  
10 transformed into various delivery vehicles for use in pest management programs.

For purposes of the present invention, pests include but are not limited to insects, fungi, bacteria, nematodes, mites, ticks, protozoan pathogens, animal-parasitic liver flukes, and the like. Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera,  
15 Isoptera, Anoplura, Siphonaptera, Trichoptera, etc.

Tables 1 - 10 gives a list of pests associated with major crop plants and pests of human and veterinary importance. Such pests are included within the scope of the present invention.

**TABLE 1**  
**Lepidoptera (Butterflies and Moths)**

5	Maize		Sunflower
	<u>Ostrinia nubilalis</u> , European corn borer	35	<u>Suleima helianthana</u> , sunflower bud moth
	<u>Agrotis ipsilon</u> , black cutworm		<u>Homoeosoma electellum</u> , sunflower moth
	<u>Helicoverpa zea</u> , corn earworm		
10	<u>Spodoptera frugiperda</u> , fall armyworm		Cotton
	<u>Diatraea grandiosella</u> , southwestern corn borer	40	<u>Heliothis virescens</u> , cotton boll worm
	<u>Elasmopalpus lignosellus</u> , lesser cornstalk borer		<u>Helicoverpa zea</u> , cotton bollworm
15	<u>Diatraea saccharalis</u> , sugarcane borer		<u>Spodoptera exigua</u> , beet armyworm
			<u>Pectinophora gossypiella</u> , pink bollworm
	Sorghum	45	Rice
	<u>Chilo partellus</u> , sorghum borer		<u>Diatraea saccharalis</u> , sugarcane borer
	<u>Spodoptera frugiperda</u> , fall armyworm		<u>Spodoptera frugiperda</u> , fall armyworm
20	<u>Helicoverpa zea</u> , corn earworm		<u>Helicoverpa zea</u> , corn earworm
	<u>Elasmopalpus lignosellus</u> , lesser cornstalk borer	50	Soybean
	<u>Feltia subterranea</u> , granulate cutworm		<u>Pseudoplusia includens</u> , soybean looper
25	Wheat		<u>Anticarsia gemmatilis</u> , velvetbean caterpillar
	<u>Pseudaletia unipunctata</u> , army worm		<u>Plathypena scabra</u> , green cloverworm
	<u>Spodoptera frugiperda</u> , fall armyworm	55	<u>Ostrinia nubilalis</u> , European corn borer
	<u>Elasmopalpus lignosellus</u> , lesser cornstalk borer		<u>Agrotis ipsilon</u> , black cutworm
30	<u>Agrotis orthogonia</u> , pale western cutworm		<u>Spodoptera exigua</u> , beet armyworm
	<u>Elasmopalpus lignosellus</u> , lesser cornstalk borer	60	<u>Heliothis virescens</u> , cotton boll worm
			<u>Helicoverpa zea</u> , cotton bollworm
			Barley
			<u>Ostrinia nubilalis</u> , European corn borer
			<u>Agrotis ipsilon</u> , black cutworm

TABLE 2

Coleoptera (Beetles)

Maize	
5	<u>Diabrotica virgifera virgifera</u> , western corn rootworm <u>Diabrotica longicornis barberi</u> , northern corn rootworm <u>Diabrotica undecimpunctata howardi</u> , southern corn rootworm <u>Melanotus</u> spp., wireworms <u>Cyclocephala borealis</u> , northern masked chafer (white grub)
10	<u>Cyclocephala immaculata</u> , southern masked chafer (white grub) <u>Popillia japonica</u> , Japanese beetle <u>Chaetocnema pulicaria</u> , corn flea beetle <u>Sphenophorus maidis</u> , maize billbug
Sorghum	
15	<u>Phyllophaga crinita</u> , white grub <u>Eleodes</u> , <u>Conoderus</u> , and <u>Aeolus</u> spp., wireworms <u>Oulema melanopus</u> , cereal leaf beetle <u>Chaetocnema pulicaria</u> , corn flea beetle
20	<u>Sphenophorus maidis</u> , maize billbug
Wheat	
	<u>Oulema melanopus</u> , cereal leaf beetle <u>Hypera punctata</u> , clover leaf weevil
25	<u>Diabrotica undecimpunctata howardi</u> , southern corn rootworm
Sunflower	
	<u>Zygogramma exclamationis</u> , sunflower beetle <u>Bothyrus gibbosus</u> , carrot beetle
30	
Cotton	
	<u>Anthonomus grandis</u> , boll weevil
Rice	
35	<u>Colaspis brunnea</u> , grape colaspis <u>Lissorhoptrus oryzophilus</u> , rice water weevil <u>Sitophilus oryzae</u> , rice weevil
Soybean	
40	<u>Epilachna varivestis</u> , Mexican bean beetle

TABLE 3

Homoptera (Whiteflies, Aphids etc..)

5	Maize	<u>Rhopalosiphum maidis</u> , corn leaf aphid <u>Anuraphis maidiradicis</u> , com root aphid
	Sorghum	
10		<u>Rhopalosiphum maidis</u> , corn leaf aphid <u>Sipha flava</u> , yellow sugarcane aphid
	Wheat	
15		Russian wheat aphid <u>Schizaphis graminum</u> , greenbug <u>Macrosiphum avenae</u> , English grain aphid
	Cotton	
20		<u>Aphis gossypii</u> , cotton aphid <u>Pseudatomoscelis seriatus</u> , cotton fleahopper <u>Trialeurodes abutilonea</u> , bandedwinged whitefly
	Rice	
25		<u>Nephotettix nigropictus</u> , rice leafhopper
	Soybean	
		<u>Myzus persicae</u> , green peach aphid <u>Empoasca fabae</u> , potato leafhopper
30	Barley	<u>Schizaphis graminum</u> , greenbug
	Oil Seed Rape	
		<u>Brevicoryne brassicae</u> , cabbage aphid



TABLE 4

Hemiptera (Bugs)

5	Maize	<u>Blissus leucopterus leucopterus</u> , chinch bug
	Sorghum	<u>Blissus leucopterus leucopterus</u> , chinch bug
10	Cotton	<u>Lygus lineolaris</u> , tarnished plant bug
	Rice	<u>Blissus leucopterus leucopterus</u> , chinch bug <u>Acrosternum hilare</u> , green stink bug
15	Soybean	<u>Acrosternum hilare</u> , green stink bug
20	Barley	<u>Blissus leucopterus leucopterus</u> , chinch bug <u>Acrosternum hilare</u> , green stink bug <u>Euschistus servus</u> , brown stink bug

TABLE 5

Orthoptera (Grasshoppers, Crickets, and Cockroaches)

5	Maize	<u>Melanoplus femurrubrum</u> , redlegged grasshopper
		<u>Melanoplus sanguinipes</u> , migratory grasshopper
10	Wheat	<u>Melanoplus femurrubrum</u> , redlegged grasshopper
		<u>Melanoplus differentialis</u> , differential grasshopper
		<u>Melanoplus sanguinipes</u> , migratory grasshopper
15	Cotton	<u>Melanoplus femurrubrum</u> , redlegged grasshopper
		<u>Melanoplus differentialis</u> , differential grasshopper
20	Soybean	<u>Melanoplus femurrubrum</u> , redlegged grasshopper
		<u>Melanoplus differentialis</u> , differential grasshopper
	Structural/Household	<u>Periplaneta americana</u> , American cockroach
		<u>Blattella germanica</u> , German cockroach
		<u>Blatta orientalis</u> , oriental cockroach

TABLE 6

Diptera (Flies and Mosquitoes)

5	Maize <u>Hylemya platura</u> , seedcorn maggot <u>Agromyza parvicornis</u> , corn blotch leafminer
10	Sorghum <u>Contarinia sorghicola</u> , sorghum midge
15	Wheat <u>Mayetiola destructor</u> , Hessian fly <u>Sitodiplosis mosellana</u> , wheat midge <u>Meromyza americana</u> , wheat stem maggot <u>Hylemya coarctata</u> , wheat bulb fly
20	Sunflower <u>Neolasioptera murtfeldtiana</u> , sunflower seed midge
25	Soybean <u>Hylemya platura</u> , seedcorn maggot
30	Barley <u>Hylemya platura</u> , seedcorn maggot <u>Mayetiola destructor</u> , Hessian fly
35	Insects attacking humans and animals and disease carriers <u>Aedes aegypti</u> , yellowfever mosquito <u>Aedes albopictus</u> , forest day mosquito <u>Phlebotomus papatasi</u> , sand fly <u>Musca domestica</u> , house fly <u>Tabanus atratus</u> , black horse fly <u>Cochliomyia hominivorax</u> , screwworm fly

**TABLE 7**Thysanoptera (Thrips)

5	Maize	
		<u>Anaphothrips obscurus</u> , grass thrips
	Wheat	
		<u>Frankliniella fusca</u> , tobacco thrips
10	Cotton	
		<u>Thrips tabaci</u> , onion thrips
		<u>Frankliniella fusca</u> , tobacco thrips
15	Soybean	
		<u>Sericothrips variabilis</u> , soybean thrips
		<u>Thrips tabaci</u> , onion thrips

20

**TABLE 8**Hymenoptera (Sawflies, Ants, Wasps, etc.)

25	Maize	
		<u>Solenopsis milesta</u> , thief ant
	Wheat	
		<u>Cephus cinctus</u> , wheat stem sawfly

TABLE 9

Other Orders and Representative Species

- Dermaptera (Earwigs)  
5      Forficula auricularia, European earwig
- Isoptera (Termites)  
      Reticulitermes flavipes, eastern subterranean termite
- 10    Mallophaga (Chewing Lice)  
      Cuclotogaster heterographa, chicken head louse  
      Bovicola bovis, cattle biting louse
- 15    Anoplura (Sucking Lice)  
      Pediculus humanus, head and body louse
- Siphonaptera (Fleas)  
      Ctenocephalides felis, cat flea

TABLE 10

Acari (Mites and Ticks)

5	Maize <u>Tetranychus urticae</u> , twospotted spider mite
10	Sorghum <u>Tetranychus cinnabarinus</u> , carmine spider mite <u>Tetranychus urticae</u> , twospotted spider mite
	Wheat <u>Aceria tulipae</u> , wheat curl mite
15	Cotton <u>Tetranychus cinnabarinus</u> , carmine spider mite <u>Tetranychus urticae</u> , twospotted spider mite
20	Soybean <u>Tetranychus turkestanii</u> , strawberry spider mite <u>Tetranychus urticae</u> , twospotted spider mite
	Barley <u>Petrobia latens</u> , brown wheat mite
25    30	Important human and animal <u>Acari</u> <u>Dermacentor variabilis</u> , American dog tick <u>Argas persicus</u> , fowl tick <u>Dermatophagoides farinae</u> , American house dust mite <u>Dermatophagoides pteronyssinus</u> , European house dust mite

Now that it has been recognized that pesticidal proteins can be isolated from the vegetative growth phase of Bacillus, other strains can be isolated by standard techniques and tested for activity against particular plant and non-plant pests. Generally Bacillus strains can be isolated from any environmental sample, including soil, plant, insect, grain elevator dust, and other sample material, etc., by methods known in the art. See, for example, Travers et al. (1987) Appl. Environ. Microbiol. 53:1263-1266; Saleh et al. (1969) Can J. Microbiol. 15:1101-1104; DeLucca et al. (1981) Can J. Microbiol. 27:865-870; and Norris, et al. (1981) "The genera Bacillus and Sporolactobacillus," In Starr et al. (eds.), The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria, Vol. II, Springer-Verlog Berlin Heidelberg.

10 After isolation, strains can be tested for pesticidal activity during vegetative growth. In this manner, new pesticidal proteins and strains can be identified.

Such Bacillus microorganisms which find use in the invention include Bacillus cereus and Bacillus thuringiensis, as well as those Bacillus species listed in Table 11.

TABLE 11

List of Bacillus species

	Morphological Group 1		Unassigned Strains
5	<u>B. megaterium</u>		Subgroup A
	<u>B. cereus*</u>		<u>B. apiarus*</u>
	<u>B. cereus var. mycoides</u>	35	<u>B. filicolonicus</u>
	<u>B. thuringiensis*</u>		<u>B. thiaminolyticus</u>
	<u>B. licheniformis</u>		<u>B. alcalophilus</u>
10	<u>B. subtilis*</u>		
	<u>B. pumilus</u>		Subgroup B
	<u>B. firmus*</u>	40	<u>B. cirroflagellosus</u>
	<u>B. coagulans</u>		<u>B. chitinosporus</u>
			<u>B. lentus</u>
15	Morphological Group 2		Subgroup C
	<u>B. polymyxa</u>		<u>B. badius</u>
	<u>B. macerans</u>	45	<u>B. aneurinolyticus</u>
	<u>B. circulans</u>		<u>B. macroides</u>
	<u>B. stearothermophilus</u>		<u>B. freundenreichii</u>
20	<u>B. alvei*</u>		
	<u>B. laterosporus*</u>		Subgroup D
	<u>B. brevis</u>	50	<u>B. pantothenicus</u>
	<u>B. pulvifaciens</u>		<u>B. epiphytus</u>
	<u>B. popilliae*</u>		
25	<u>B. lentimorbus*</u>		Subgroup E1
	<u>B. larvae*</u>	55	<u>B. aminovorans</u>
			<u>B. globisporus</u>
	Morphological Group 3		<u>B. insolitus</u>
30	<u>B. sphaericus*</u>		<u>B. psychrophilus</u>
	<u>B. pasteurii</u>		
		60	Subgroup E2
			<u>B. psychrosaccharolyticus</u>
			<u>B. macquariensis</u>

\*=Those Bacillus strains that have been previously found in insects

65 Grouping according to Parry, J.M. et al. (1983) Color Atlas of Bacillus species, Wolfe Medical Publications, London.



In accordance with the present invention, the pesticidal proteins produced during vegetative growth can be isolated from Bacillus. In one embodiment, insecticidal proteins produced during vegetative growth, herein after referred to as VIP's (Vegetative Insecticidal Protein), can be isolated. Methods for protein isolation are known in the art. Generally, proteins  
5 can be purified by conventional chromatography, including gel-filtration, ion-exchange, and immunoaffinity chromatography, by high-performance liquid chromatography, such as reversed-phase high-performance liquid chromatography, ion-exchange high-performance liquid chromatography, size-exclusion high-performance liquid chromatography, high-performance chromatofocusing and hydrophobic interaction chromatography, etc., by electrophoretic  
10 separation, such as one-dimensional gel electrophoresis, two-dimensional gel electrophoresis, etc. Such methods are known in the art. See for example Current Protocols in Molecular Biology, Vols. 1 and 2, Ausubel et al. (eds.), John Wiley & Sons, NY (1988). Additionally, antibodies can be prepared against substantially pure preparations of the protein. See, for example, Radka et al. (1983) J. Immunol. 128:2804; and Radka et al. (1984) Immunogenetics  
15 19:63. Any combination of methods may be utilized to purify protein having pesticidal properties. As the protocol is being formulated, pesticidal activity is determined after each purification step.

Such purification steps will result in a substantially purified protein fraction. By "substantially purified" or "substantially pure" is intended protein which is substantially free of  
20 any compound normally associated with the protein in its natural state. "Substantially pure" preparations of protein can be assessed by the absence of other detectable protein bands following SDS-PAGE as determined visually or by densitometry scanning. Alternatively, the absence of other amino-terminal sequences or N-terminal residues in a purified preparation can indicate the level of purity. Purity can be verified by rechromatography of "pure" preparations  
25 showing the absence of other peaks by ion exchange, reverse phase or capillary electrophoresis.

The terms "substantially pure" or "substantially purified" are not meant to exclude artificial or synthetic mixtures of the proteins with other compounds. The terms are also not meant to exclude the presence of minor impurities which do not interfere with the biological activity of the protein, and which may be present, for example, due to incomplete purification.

5        Some proteins are single polypeptide chains while many proteins consist of more than one polypeptide chain. Once purified protein is isolated, the protein, or the polypeptides of which it is comprised, can be characterized and sequenced by standard methods known in the art. For example, the purified protein, or the polypeptides of which it is comprised, may be fragmented as with cyanogen bromide, or with proteases such as papain, chymotrypsin, trypsin, lysyl-C endopeptidase, etc. (Oike et al. (1982) J. Biol. Chem. 257:9751-9758; Liu et al. (1983) Int. J. Pept. Protein Res. 21:209-215). The resulting peptides are separated, preferably by HPLC, or by resolution of gels and electroblotting onto PVDF membranes, and subjected to amino acid sequencing. To accomplish this task, the peptides are preferably analyzed by automated sequenators. It is recognized that N-terminal, C-terminal, or internal amino acid  
10        sequences can be determined. From the amino acid sequence of the purified protein, a nucleotide sequence can be synthesized which can be used as a probe to aid in the isolation of the gene encoding the pesticidal protein.  
15

It is recognized that the proteins will vary in molecular weight, component peptides, activity against particular pests, and in other characteristics. However, by the methods set forth  
20        herein, proteins active against a variety of pests may be isolated and characterized.

Once the purified protein has been isolated and characterized it is recognized that it may be altered in various ways including amino acid substitutions, deletions, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the pesticidal proteins can be prepared by mutations in the DNA. Such variants will  
25        possess the desired pesticidal activity. Obviously, the mutations that will be made in the DNA

encoding the variant must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See, EP Patent Application Publication No. 75,444.

In this manner, the present invention encompasses the pesticidal proteins as well as  
5 components and fragments thereof. That is, it is recognized that component polypeptides or fragments of the proteins may be produced which retain pesticidal activity. These fragments include truncated sequences, as well as N-terminal, C-terminal, internal and internally deleted amino acid sequences of the proteins.

Most deletions, insertions, and substitutions of the protein sequence are not expected to  
10 produce radical changes in the characteristics of the pesticidal protein. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays.

The proteins or other component polypeptides described herein may be used alone or in  
15 combination. That is, several proteins may be used to control different insect pests. Additionally, certain of the proteins of the invention enhance the activity of the pesticidal proteins. These proteins are referred to herein as "auxiliary proteins." While the mechanism of action is not entirely certain, when the auxiliary protein and the pesticidal protein of interest are together, the insecticidal properties of the pesticidal protein are enhanced several fold.

20 The pesticidal proteins of the present invention may vary in molecular weight, having component polypeptides at least a molecular weight of 30 kDa or greater, preferably about 50 kDa or greater.

The auxiliary proteins of the invention may vary in molecular weight, having at least a molecular weight of about 15 kDa or greater, preferably about 20 kDa or greater. The auxiliary  
25 proteins themselves may have component polypeptides.

It is possible that the pesticidal protein and the auxiliary protein may be components of a multimeric, pesticidal protein. Such a pesticidal protein which includes the auxiliary proteins as one or more of its component polypeptides may vary in molecular weight, having at least a molecular weight of 50 kDa up to at least 200 kDa, preferably about 100 kDa to 150 kDa.

5        An auxiliary protein may be used in combination with the pesticidal proteins of the invention to enhance activity. To determine whether the auxiliary protein will affect activity, the pesticidal protein can be expressed alone and in combination with the auxiliary protein and the respective activities compared in feeding assays for increased pesticidal activity.

10       It may be beneficial to screen strains for potential pesticidal activity by testing activity of the strain alone and in combination with the auxiliary protein. In some instances the auxiliary protein with the native proteins of the strains yields pesticidal activity where none is seen in the absence of the auxiliary protein.

15       The auxiliary protein can be modified, as described above, by various methods known in the art. Therefore, for purposes of the invention, the term "Vegetative Insecticidal Protein" (VIP) encompasses those proteins produced during vegetative growth which alone or in combination can be used for pesticidal activity. This includes pesticidal proteins, auxiliary proteins and those proteins which demonstrate activity only in the presence of the auxiliary protein or the polypeptide components of these proteins.

20       It is recognized that there are alternative methods available to obtain the nucleotide and amino acid sequences of the present proteins. For example, to obtain the nucleotide sequence encoding the pesticidal protein, cosmid clones, which express the pesticidal protein, can be isolated from a genomic library. From larger active cosmid clones, smaller subclones can be made and tested for activity. In this manner, clones which express an active pesticidal protein can be sequenced to determine the nucleotide sequence of the gene. Then, an amino acid  
25       sequence can be deduced for the protein. For general molecular methods, see, for example,

Molecular Cloning, A Laboratory Manual, Second Edition, Vols. 1-3, Sambrook et al. (eds.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), and the references cited therein.

The present invention also encompasses nucleotide sequences from organisms other than  
5 Bacillus, where the nucleotide sequences are isolatable by hybridization with the Bacillus  
nucleotide sequences of the invention. Such nucleotide sequences can be tested for pesticidal  
activity. The invention also encompasses the proteins encoded by the nucleotide sequences.  
Furthermore, the invention encompasses proteins obtained from organisms other than Bacillus  
wherein the protein cross-reacts with antibodies raised against the proteins of the invention.  
10 Again the isolated proteins can be assayed for pesticidal activity by the methods disclosed  
herein.

Once the nucleotide sequences encoding the pesticidal proteins of the invention have  
been isolated, they can be manipulated and used to express the protein in a variety of hosts  
including other organisms, including microorganisms and plants.

15 The pesticidal genes of the invention can be optimized for enhanced expression in plants.  
See, for example U.S. Application Serial No. 07/951,715; EPA 0359472; EPA 0385962; WO  
91/16432; Perlak et al (1991) Proc. Natl. Acad. Sci. USA 88:3324-3328; and Murray et al  
(1989) Nucleic Acids Research 17: 477-498. In this manner, the genes can be synthesized  
utilizing plant preferred codons. That is the preferred codon for a particular host is the single  
20 codon which most frequently encodes that amino acid in that host. The maize preferred codon,  
for example, for a particular amino acid may be derived from known gene sequences from  
maize. Maize codon usage for 28 genes from maize plants is found in Murray et al. (1989),  
Nucleic Acids Research 17:477-498, the disclosure of which is incorporated herein by reference.  
Synthetic genes could also be made based on the distribution of codons a particular host uses for  
25 a particular amino acid.

In this manner, the nucleotide sequences can be optimized for expression in any plant. It is recognized that all or any part of the gene sequence may be optimized or synthetic. That is, synthetic or partially optimized sequences may also be used.

In like manner, the nucleotide sequences can be optimized for expression in any  
5 microorganism. For Bacillus preferred codon usage, see, for example US Patent No. 5,024,837 and Johansen et al (1988) Gene 65:293-304.

Methodologies for the construction of plant expression cassettes as well as the introduction of foreign DNA into plants are described in the art. Such expression cassettes may include promoters, terminators, enhancers, leader sequences, introns and other regulatory  
10 sequences operably linked to the pesticidal protein coding sequence.

Generally, for the introduction of foreign DNA into plants Ti plasmid vectors have been utilized for the delivery of foreign DNA as well as direct DNA uptake, liposomes, electroporation, micro-injection, and the use of microprojectiles. Such methods had been published in the art. See, for example, Guerche et al., (1987) Plant Science 52:111-116;  
15 Neuhauser et al., (1987) Theor. Appl. Genet. 75:30-36; Klein et al., (1987) Nature 327: 70-73; Howell et al., (1980) Science 208:1265; Horsch et al., (1985) Science 227: 1229-1231; DeBlock et al., (1989) Plant Physiology 91:694-701; Methods for Plant Molecular Biology (Weissbach and Weissbach, eds.) Academic Press, Inc. (1988); and Methods in Plant Molecular Biology (Schuler and Zielinski, eds.) Academic Press, Inc. (1989). See also US patent Application Serial  
20 No. 08/008,374 herein incorporated by reference. See also, EPA 0193259 and EPA 0451878A1. It is understood that the method of transformation will depend upon the plant cell to be transformed.

It is further recognized that the components of the expression cassette may be modified to increase expression. For example, truncated sequences, nucleotide substitutions or other  
25 modifications may be employed. See, for example Perlak et al. (1991) Proc. Natl. Acad. Sci.

USA 88:3324-3328; Murray et al. (1989) Nucleic Acids Research 17:477-498; and WO 91/16432.

The construct may also include any other necessary regulators such as terminators, (Guerineau et al., (1991), Mol. Gen. Genet., 226:141-144; Proudfoot, (1991), Cell, 5 64:671-674; Sanfacon et al., (1991), Genes Dev., 5:141-149; Mogen et al., (1990), Plant Cell, 2:1261-1272; Munroe et al., (1990), Gene, 91:151-158; Ballas et al., (1989), Nucleic Acids Res., 17:7891-7903; Joshi et al., (1987), Nucleic Acid Res., 15:9627-9639); plant translational consensus sequences (Joshi, C.P., (1987), Nucleic Acids Research, 15:6643-6653), introns (Luehrsen and Walbot, (1991), Mol. Gen. Genet., 225:81-93) and the like, operably linked to the 10 nucleotide sequence. It may be beneficial to include 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translational leaders are known in the art and include:

Picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein, O., Fuerst, T.R., and Moss, B. (1989) PNAS USA 86:6126-6130);

15 Potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Allison et al., (1986); MDMV leader (Maize Dwarf Mosaic Virus); Virology, 154:9-20), and

Human immunoglobulin heavy-chain binding protein (BiP), (Macejak, D.G., and Sarnow, P., (1991), Nature, 353:90-94;

Untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4), 20 (Jobling, S.A., and Gehrke, L., (1987), Nature, 325:622-625;

Tobacco mosaic virus leader (TMV), (Gallie, D.R. et al., (1989), Molecular Biology of RNA, pages 237-256; and

Maize Chlorotic Mottle Virus leader (MCMV) (Lommel, S.A. et al., (1991), Virology, 81:382-385. See also, Della-Cioppa et al., (1987), Plant Physiology, 84:965-968.

A plant terminator may be utilized in the expression cassette. See, Rosenberg et al., (1987), Gene, 56:125; Guerineau et al., (1991), Mol. Gen. Genet., 226:141-144; Proudfoot, (1991), Cell, 64:671-674; Sanfacon et al., (1991), Genes Dev., 5:141-149; Mogen et al., (1990), Plant Cell, 2:1261-1272; Munroe et al., (1990), Gene, 91:151-158; Ballas et al., (1989), Nucleic  
5 Acids Res., 17:7891-7903; Joshi et al., (1987), Nucleic Acid Res., 15:9627-9639.

For tissue specific expression, the nucleotide sequences of the invention can be operably linked to tissue specific promoters. See, for example, US Application Serial No. 07/951,715 herein incorporated by reference.

It is recognized that the genes encoding the pesticidal proteins can be used to transform  
10 insect pathogenic organisms. Such organisms include Baculoviruses, fungi, protozoa, bacteria and nematodes.

The Bacillus strains of the invention may be used for protecting agricultural crops and products from pests. Alternatively, a gene encoding the pesticide may be introduced via a suitable vector into a microbial host, and said host applied to the environment or plants or  
15 animals. Microorganism hosts may be selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplana) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for  
20 improved protection of the pesticide from environmental degradation and inactivation.

Such microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., Pseudomonas, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Rhizobium, Rhodopseudomonas, Methylius, Agrobacterium, Acetobacter, Lactobacillus, Arthrobacter, Azotobacter, Leuconostoc, and Alcaligenes; fungi,  
25 particularly yeast, e.g., Saccharomyces, Cryptococcus, Kluyveromyces, Sporobolomyces,



Rhodotorula, and Aureobasidium. Of particular interest are such phytosphere bacterial species as Pseudomonas syringae, Pseudomonas fluorescens, Serratia marcescens, Acetobacter xylinum, Agrobacteria, Rhodopseudomonas spheroides, Xanthomonas campestris, Rhizobium melioli, Alcaligenes entrophus, Clavibacter xyli and Azotobacter vinlandii; and phytosphere yeast species such as Rhodotorula rubra, R. glutinis, R. marina, R. aurantiaca, Cryptococcus albidus, C. diffluens, C. laurentii, Saccharomyces rosei, S. pretoriensis, S. cerevisiae, Sporobolomyces rosues, S. odoris, Kluyveromyces veronae, and Aureobasidium pollulans. Of particular interest are the pigmented microorganisms.

A number of ways are available for introducing a gene expressing the pesticidal protein into the microorganism host under conditions which allow for stable maintenance and expression of the gene. For example, expression cassettes can be constructed which include the DNA constructs of interest operably linked with the transcriptional and translational regulatory signals for expression of the DNA constructs, and a DNA sequence homologous with a sequence in the host organism, whereby integration will occur, and/or a replication system which is functional in the host, whereby integration or stable maintenance will occur.

Transcriptional and translational regulatory signals include but are not limited to promoter, transcriptional initiation start site, operators, activators, enhancers, other regulatory elements, ribosomal binding sites, an initiation codon, termination signals, and the like. See, for example, US Patent 5,039,523; US Patent No. 4,853,331; EPO 0480762A2; Sambrook et al. supra; Molecular Cloning, a Laboratory Manual, Maniatis et al. (eds) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982); Advanced Bacterial Genetics, Davis et al. (eds.) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1980); and the references cited therein.

Suitable host cells, where the pesticide-containing cells will be treated to prolong the activity of the toxin in the cell when the then treated cell is applied to the environment of the

target pest(s), may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxin is unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and -positive, include Enterobacteriaceae, such as Escherichia, Erwinia, Shigella, Salmonella, and Proteus; Bacillaceae; Rhizobiceae, such as Rhizobium; Spirillaceae, such as photobacterium, Zymomonas, Serratia, Aeromonas, Vibrio, Desulfovibrio, Spirillum; Lactobacillaceae; Pseudomonadaceae, such as Pseudomonas and Acetobacter; Azotobacteraceae and Nitrobacteraceae. Among eukaryotes are fungi, such as Phycomycetes and Ascomycetes, which includes yeast, such a Saccharomyces and Schizosaccharomyces; and Basidiomycetes yeast, such as Rhodotorula, Aureobasidium, Sporobolomyces, and the like.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the protein gene into the host, availability of expression systems, efficiency of expression, stability of the protein in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Host organisms of particular interest include yeast, such as Rhodotorula sp., Aureobasidium sp., Saccharomyces sp., and Sporobolomyces sp.; phylloplane organisms such as Pseudomonas sp., Erwinia sp. and Flavobacterium sp.; or such other organisms as Escherichia,

Lactobacillus sp., Bacillus sp., and the like. Specific organisms include Pseudomonas  
aeruginosa, Pseudomonas fluorescens, Saccharomyces cerevisiae, Bacillus thuringiensis,  
Escherichia coli, Bacillus subtilis, and the like.

General methods for employing the strains of the invention in pesticide control or in  
5 engineering other organisms as pesticidal agents are known in the art. See, for example US  
Patent No. 5,039,523 and EP 0480762A2.

The Bacillus strains of the invention or the microorganisms which have been genetically  
altered to contain the pesticidal gene and protein may be used for protecting agricultural crops  
and products from pests. In one aspect of the invention, whole, i.e., unlysed, cells of a toxin  
10 (pesticide)-producing organism are treated with reagents that prolong the activity of the toxin  
produced in the cell when the cell is applied to the environment of target pest(s).

Alternatively, the pesticides are produced by introducing a heterologous gene into a  
cellular host. Expression of the heterologous gene results, directly or indirectly, in the  
intracellular production and maintenance of the pesticide. These cells are then treated under  
15 conditions that prolong the activity of the toxin produced in the cell when the cell is applied to  
the environment of target pest(s). The resulting product retains the toxicity of the toxin. These  
naturally encapsulated pesticides may then be formulated in accordance with conventional  
techniques for application to the environment hosting a target pest, e.g., soil, water, and foliage  
of plants. See, for example EPA 0192319, and the references cited therein.

20 The active ingredients of the present invention are normally applied in the form of  
compositions and can be applied to the crop area or plant to be treated, simultaneously or in  
succession, with other compounds. These compounds can be both fertilizers or micronutrient  
donors or other preparations that influence plant growth. They can also be selective herbicides,  
insecticides, fungicides, bactericides, nematocides, molluscicides or mixtures of several of these  
25 preparations, if desired, together with further agriculturally acceptable carriers, surfactants or

application-promoting adjuvants customarily employed in the art of formulation. Suitable carriers and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g. natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders or fertilizers.

5 Preferred methods of applying an active ingredient of the present invention or an agrochemical composition of the present invention which contains at least one of the pesticidal proteins produced by the bacterial strains of the present invention are leaf application, seed coating and soil application. The number of applications and the rate of application depend on the intensity of infestation by the corresponding pest.

10 In one embodiment of the invention a Bacillus cereus microorganism has been isolated which is capable of killing Diabrotica virgifera virgifera, and Diabrotica longicornis barberi. The novel B. cereus strain AB78 has been deposited in the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, IL 61604, USA and given Accession No. NRRL B-21058.

15 A protein has been substantially purified from the B. cereus strain. Purification of the protein has been verified by SDS-PAGE and biological activity. The protein has a molecular weight of about 60 to about 100 kDa, particularly about 70 to about 90 kDa, more particularly about 80 kDa.

Amino-terminal sequencing has revealed the N-terminal amino-acid sequence to be:

20 NH<sub>2</sub>-Lys-Arg-Glu-Ile-Asp-Glu-Asp-Thr-Asp-Thr-Asx-Gly-Asp-Ser-Ile-Pro- (SEQ ID NO:8)  
where Asx represents either Asp or Asn. The entire amino acid sequence is given in SEQ ID NO:7.

An oligonucleotide probe for the region of the gene encoding amino acids 3-9 of the NH<sub>2</sub>-terminus has been generated. The probe was synthesized based on the codon usage of a

Bacillus thuringensis (Bt)  $\delta$ -endotoxin gene. The nucleotide sequence of the oligonucleotide probe used for Southern hybridizations was as follows:

5'- GAA ATT GAT CAA GAT ACN GAT -3' (SEQ ID NO:9)

where N represents any base.

- 5 In addition, the DNA probe for the Bc AB78 VIP-1 gene described herein, permits the screening of any Bacillus strain or other organisms to determine whether the VIP-1 gene (or related gene) is naturally present or whether a particular transformed organism includes the VIP-1 gene.

- 10 The invention now being generally described, the same will be better understood by reference to the following detailed examples that are provided for the purpose of illustration and are not to be considered limiting of the invention unless so specified.

EXPERIMENTAL**Example 1. AB78 Isolation and Characterization**

**Bacillus cereus** strain AB78 was isolated as a plate contaminant in the laboratory on T3 media (per liter: 3 g tryptone, 2 g tryptose, 1.5 g yeast extract, 0.05 M sodium phosphate (pH 6.8), and 0.005 g MnCl<sub>2</sub>; Travers, R.S. 1983). AB78 gave significant activity against western corn rootworm. Antibiotic activity against gram-positive Bacillus spp. was also demonstrated (Table 12).

**Table 12**Antibiotic activity of AB78 culture supernatant

<u>Bacteria tested</u>	<u>Zone of inhibition(cm)</u>	
	<u>AB78</u>	<u>Streptomycin</u>
<u>E. coli</u>	0.0	3.0
<u>B. megaterium</u>	1.1	2.2
<u>B. mycoides</u>	1.3	2.1
<u>B. cereus</u> CB	1.0	2.0
<u>B. cereus</u> 11950	1.3	2.1
<u>B. cereus</u> 14579	1.0	2.4
<u>B. cereus</u> AB78	0.0	2.2
<u>Bt var. israelensis</u>	1.1	2.2
<u>Bt var. tenebrionis</u>	0.9	2.3

Morphological characteristics of AB78 are as follows:

Vegetative rods straight, 3.1-5.0 mm long and 0.5-2.0 mm wide. Cells with rounded ends, single in short chains. Single subterminal, cylindrical-oval, endospore formed per cell. No

parasporal crystal formed. Colonies opaque, erose, lobate and flat. No pigments produced.

Cells motile. Flagella present.

Growth characteristics of AB78 are as follows:

Facultative anaerobe with optimum growth temperature of 21-30°C. Will grow at 15,  
5 20, 25, 30 and 37°C. Will not grow above 40°C. Grows in 5-7% NaCl.

Table 13 provides the biochemical profile of AB78.

Table 13

10 Biochemical characteristics of B. cereus strain AB78.

15	Acid from L-arabinose	-	Methylene blue reoxidized	+
	Gas from L-arabinose	-	Nitrate reduced	+
	Acid from D-xylose	-	NO <sub>3</sub> reduced to NO <sub>2</sub>	+
	Gas from D-xylose	-	VP	+
	Acid from D-glucose	+	H <sub>2</sub> O <sub>2</sub> decomposed	+
	Gas from D-glucose	-	Indole	-
20	Acid from lactose	-	Tyrosine decomposed	+
	Gas from lactose	-	Dihydroxiacetone	-
	Acid from sucrose	-	Litmus milk acid	-
	Gas from sucrose	-	Litmus milk coagulated	-
	Acid from D-mannitol	-	Litmus milk alkaline	-
25	Gas from D-mannitol	-	Litmus milk peptonized	-
	Propionate utilization	+	Litmus milk reduced	-
	Citrate utilization	+	Casein hydrolyzed	+
	Hippurate hydrolysis	w	Starch hydrolyzed	+
30	Methylene blue reduced	+	Gelatin liquidified	+
			Lecithinase produced	w

w= weak reaction

**Example 2. Bacterial Culture**

A subculture of Bc strain AB78 was used to inoculate the following medium, known as TB broth:

5	Tryptone	12	g/l
	Yeast Extract	24	g/l
	Glycerol	4	ml/l
	KH <sub>2</sub> PO <sub>4</sub>	2.1	g/l
10	K <sub>2</sub> HPO <sub>4</sub>	14.7	g/l
	pH 7.4		

The potassium phosphate was added to the autoclaved broth after cooling. Flasks were incubated at 30°C on a rotary shaker at 250 rpm for 24 h.-36 h.

15 The above procedure can be readily scaled up to large fermentors by procedures well known in the art.

During vegetative growth, usually 24-36 h. after starting the culture, AB78 bacteria were centrifuged from the culture supernatant. The culture supernatant containing the active protein was used in bioassays.

20

**Example 3. Insect Bioassays**

B. cereus strain AB78 was tested against various insects as described below.

25 Western, Northern and Southern corn rootworm, Diabrotica virgifera virgifera, D. longcornis barberi and D. undecempunctata howardi, respectively:, dilutions were made of AB78 culture supernatant grown 24-36 h., mixed with molten artificial diet (Marrone et al.



(1985) J. of Economic Entomology 78:290-293) and allowed to solidify. Solidified diet was cut and placed in dishes. Neonate larvae were placed on the diet and held at 30°C. Mortality was recorded after 6 days.

**E. coli clone bioassay:** E. coli was grown overnight in L-Amp100 at 37°C. Ten ml  
5 culture was sonicated 3X for 20 sec each. 500 ml of sonicated culture was added to molten western corn rootworm diet.

**Colorado potato beetle Leptinotarsa decemlineata:**-dilutions in Triton X-100 (to give final concentration of 0.1% TX-100) were made of AB78 culture supernatant grown 24-36 h. Five cm<sup>2</sup> potato leaf pieces were dipped into these dilutions, air dried, and placed on moistened  
10 filter paper in plastic dishes. Neonate larvae were placed on the leaf pieces and held at 30°C. Mortality was recorded after 3-5 days.

**Yellow mealworm, Tenebrio molitor:**- dilutions were made of AB78 culture supernatant grown 24-36 h., mixed with molten artificial diet (Bioserv #F9240) and allowed to solidify. Solidified diet was cut and placed in plastic dishes. Neonate larvae were placed on the  
15 diet and held at 30°C. Mortality was recorded after 6-8 days.

**European corn borer, black cutworm, tobacco budworm, tobacco hornworm and beet armyworm; Ostrinia nubilalis, Agrotis ipsilon, Heliothis virescens, Manduca sexta and Spodoptera exigua, respectively:**-dilutions, in TX-100 (to give final concentration of 0.1% TX-100), were made of AB78 culture supernatant grown 24-36 hrs. 100 ml was pipetted onto  
20 the surface of 18 cm<sup>2</sup> of solidified artificial diet (Bioserv #F9240) and allowed to air dry. Neonate larvae were then placed onto the surface of the diet and held at 30°C. Mortality was recorded after 3-6 days.

**Northern house mosquito, Culex pipiens:**-dilutions were made of AB78 culture supernatant grown 24-36 h. 100 ml was pipetted into 10 ml water in a 30 ml plastic cup. Third

instar larvae were added to the water and held at room temperature. Mortality was recorded after 24-48 hours. The spectrum of entomocidal activity of AB78 is given in Table 14.

Table 14

5 Activity of AB78 culture supernatant against various insect species

	Insect species tested to date	Order	Activity
10	Western corn rootworm ( <u>Diabrotica virgifera</u> <u>virgifera</u> )	Col	+++
15	Northern corn rootworm ( <u>Diabrotica longicornis</u> <u>barberi</u> )	Col	+++
	Southern corn rootworm ( <u>Diabrotica undecimpunctata</u> <u>howardi</u> )	Col	-
20	Colorado potato beetle ( <u>Leptinotarsa decemlineata</u> )	Col	-
	Yellow mealworm ( <u>Tenebrio molitor</u> )	Col	-
	European corn borer ( <u>Ostrinia nubilalis</u> )	Lep	-
25	Tobacco budworm ( <u>Heliothis virescens</u> )	Lep	-
	Tobacco hornworm ( <u>Manduca sexta</u> )	Lep	-
30	Beet armyworm ( <u>Spodoptera exigua</u> )	Lep	-
	Black cutworm ( <u>Agrotis ipsilon</u> )	Lep	-
	Northern house mosquito ( <u>Culex pipiens</u> )	Dip	-

The newly discovered B. cereus strain AB78 showed a significantly different spectrum of insecticidal activity as compared to known coleopteran active  $\delta$ -endotoxins from Bt. In particular, AB78 showed more selective activity against beetles than known coleopteran-active Bt strains in that it was specifically active to Diabrotica spp. More specifically, it was most active against D. virgifera virgifera and D. longicornis barberi but not D. undecimpunctata howardi.

A number of Bacillus strains were bioassayed for activity during vegetative growth (Table 15) against western corn rootworm. The results demonstrate that AB78 is unique in that activity against western corn rootworm is not a general phenomenon.

Table 15

Activity of culture supernatants from various Bacillus spp. against western corn rootworm

	Bacillus strain	Percent WCRW mortality
	<u>B. cereus</u> AB78 (Bat.1)	100
	<u>B. cereus</u> AB78 (Bat.2)	100
	<u>B. cereus</u> (Carolina Bio.)	12
	<u>B. cereus</u> ATCC 11950	12
	<u>B. cereus</u> ATCC 14579	8
	<u>B. mycoides</u> (Carolina Bio.)	30
	<u>B. popilliae</u>	28
	<u>B. thuringiensis</u> HD135	41
	<u>B. thuringiensis</u> HD191	9
	<u>B. thuringiensis</u> GC91	4
	<u>B. thuringiensis isrealensis</u>	24
	Water Control	4

Specific activity of AB78 against western corn rootworm is provided in Table 16.

**Table 16**

5           **Activity of AB78 culture supernatant against neonate western corn rootworm**

	<u>Culture supernatant concentration (<math>\mu</math>l/ml)</u>	<u>Percent WCRW mortality</u>
10	100	100
	25	87
	10	80
	5	40
	2.5	20
15	1	6
	0	0

The  $LC_{50}$  was calculated to be 6.2  $\mu$ l of culture supernatant per ml of western corn rootworm diet.

20

**Example 4. Isolation and Purification of Corn Rootworm Active Protein from AB78.**

25           Culture media free of cells and debris was made to 70% saturation by the addition of solid ammonium sulfate i.e. (472 g/L). Dissolution was at room temperature followed by cooling in an ice bath and centrifugation at 10,000 x g for thirty minutes to pellet out the precipitated proteins.

            The supernatant was discarded and the pellet was dissolved in 1/10 the original volume with 20 mM TRIS-HCl at pH 7.5.

30           The dissolved pellet was desalted either by dialysis in 20 mM TRIS HCl pH 7.5, or passing through a desalting column.

The desalted material was titrated to pH 3.5 with 20 mM sodium citrate pH 2.5.

Following a thirty minute room temperature incubation the solution was centrifuged at 3000 X g for ten minutes. The supernatant at this stage contained the greatest amount of active protein.

Following neutralization of the pH to 7.0 the supernatant was applied to a Mono-Q,  
5 anion exchange, column equilibrated with 20 mM TRIS pH 7.5 at a flow rate of 300 mL/min. The column was developed with a stepwise and linear gradient employing 400 mM NaCl in 20 mM TRIS pH 7.5.

Bioassay of the column fractions and SDS-PAGE analysis were used to confirm the active fractions. SDS-PAGE analysis identified the biologically active protein as having a  
10 molecular weight in the range of 80 kDa.

#### **Example 5. Sequence Analysis of the Corn Rootworm Active Protein**

The 80 kDa protein isolated by SDS-PAGE was transferred to PVDF membrane and was  
15 subjected to amino-terminal sequencing as performed by repetitive Edman cycles on the ABI 470 pulsed-liquid sequencer. Transfer was carried out in 10 mM CAPS buffer with 10% methanol pH 11.0 as follows:

Incubation of the gel following electrophoresis was done in transfer buffer for five minutes.

20 ProBlott PVDF membrane was wetted with 100% MeOH briefly then equilibrated in transfer buffer.

The sandwich was arranged between foam sponges and filter paper squares with the configuration of Cathode-Gel-Membrane-Anode.

Transfer was performed at 70 V constant voltage for 1 hour.

Following transfer the membrane was rinsed with water and stained for two minutes with 0.25% Coomassie Blue R-250 in 50% MeOH.

Destaining was done with several rinses with 50% MeOH 40% water 10% acetic acid.

Following destaining the membrane was air dried prior to excision of the bands for  
5 sequence analysis. A BlottCartridge and appropriate cycles were utilized to achieve maximum efficiency and yield. Data analysis was performed using the model 610 Sequence Analysis software for identifying and quantifying the PTH-amino acid derivatives for each sequential cycle.

The N-terminal sequence was determined to be:

10 NH<sub>2</sub>-Lys-Arg-Glu-Ile-Asp-Glu-Asp-Thr-Asp-Thr-Asx-Gly-Asp-Ser-Ile-Pro- (SEQ ID NO:8)  
where Asx represents Asp or Asn.

#### **Example 6. Construction of DNA Probe**

An oligonucleotide probe for the region of the gene encoding amino acids 3-9 of the  
15 N-terminal sequence (Example 5) was generated. The probe was synthesized based on the codon usage of a *Bacillus thuringensis* (Bt)  $\delta$ -endotoxin gene. The nucleotide sequence

5'- GAA ATT GAT CAA GAT ACN GAT -3' (SEQ ID NO:9)

was used as a probe in Southern hybridizations. The oligonucleotide was synthesized using standard procedures and equipment.

20

#### **Example 7. Isoelectric Point Determination of the Corn Rootworm Active Protein**

Purified protein from step 5 of the purification process was analyzed on a 3-9 pI isoelectric focusing gel using the Phastgel electrophoresis system (Pharmacia). Standard

operating procedures for the unit were followed for both the separation and silver staining development procedures. The pI was approximated at about 4.9.

**Example 8. PCR Data On AB78**

PCR analysis (See, for example US patent Application Serial No. 08/008,006; and, Carozzi et al. (1991) Appl. Environ. Microbiol. 57(11):3057-3061, herein incorporated by reference.) was used to verify that the B. cereus strain AB78 did not contain any insecticidal crystal protein genes of B. thuringensis or B. sphaericus (Table 17).

**Table 17**

Bacillus insecticidal crystal protein gene primers tested by PCR against AB78 DNA.

	<u>Primers Tested</u>	<u>Product Produced</u>
15	2 sets specific for CryIIIA	Negative
	CryIIIB	Negative
	2 sets specific for CryIA	Negative
	CryIA(a)	Negative
	CryIA(b) specific	Negative
20	CryIB	Negative
	CryIC specific	Negative
	CryIE specific	Negative
	2 sets specific for <u>B. sphaericus</u>	Negative
	2 sets specific for CryIV	Negative
25	<u>Bacillus control (PI-PLC)</u>	<u>Positive</u>

**Example 9. Cosmid Cloning of Total DNA from B. cereus Strain AB78**

The VIP-1 gene was cloned from total DNA prepared from strain AB78 as follows:

5

**Isolation of AB78 DNA was as follows:**

1. Grow bacteria in 10 ml L-broth overnight. (Use 50 ml sterile centrifuge tube)
2. Add 25 ml of fresh L-broth and ampicillin (30 mg/ml).
3. Grow cells 2-6 h. at 30°C with shaking.
- 10 4. Spin cells in a 50 ml polypropylene orange cap tube in IEC benchtop clinical centrifuge at 3/4 speed.
5. Resuspend cell pellet in 10 ml TES.
6. Add 30 mg lysozyme and incubate 2 hrs at 37°C.
7. Add 200 ml 20% SDS and 400 ml Proteinase K (20 mg/ml). Incubate at 37°C.
- 15 8. Add 200 ml fresh Proteinase K. Incubate 1 hr. at 55°C. Add 5 ml TES (TES = 50 mM Tris pH 8.0, 100mM EDTA, 15 mM NaCl) to make 15 ml final volume.
9. Phenol extract twice (10 ml phenol, spin at room temperature at 3/4 speed in an IEC benchtop clinical centrifuge). Transfer supernatant (top) to a clean tube with a wide bore pipet.
- 20 10. Extract once with 1:1 vol. phenol:chloroform/isoamyl alcohol (24:1 ratio).
11. Precipitate DNA with an equal volume of cold isopropanol; Centrifuge to pellet DNA.
12. Resuspend pellet in 5 ml TE.
13. Precipitate DNA with 0.5 ml 3M NaOAc pH 5.2 and 11 ml 95% ethanol. Place at -20°C for 2 h.



14. "Hook" DNA from tube with a plastic loop, transfer to a microfuge tube, spin, pipet off excess ethanol, dry in vacuo.
15. Resuspend in 0.5 ml TE. Incubate 90 min. at 65°C to help get DNA back into solution.
16. Determine concentration using standard procedures.

5

**Cosmid Cloning of AB78**

All procedures, unless indicated otherwise, were performed according to Stratagene Protocol, Supercos 1 Instruction Manual, Cat. No. 251301.

Generally, the steps were as follows:

- 5       A.     Sau 3A Partial Digestion of the AB78 DNA.
- B.     Preparation of Vector DNA
- C.     Ligation and packaging of DNA
- D.     Titering the cosmid library
  - 10       1.     Start a culture of HB101 cells by placing 50 ml of an overnight culture in  
              5 mls of TB with 0.2% maltose. Incubate 3.5 hrs. at 37°C.
  2.     Spin out cells and resuspend in 0.5 mls 10 mM MgSO<sub>4</sub>.
  3.     Add together:  
              100 ml cells  
              100 ml diluted packaging mixture  
15       100 ml 10 mM MgSO<sub>4</sub>  
              30 ml TB
  4.     Adsorb at room temperature for 30 minutes with no shaking.
  5.     Add 1 ml TB and mix gently. Incubate 30 minutes at 37°C.
  6.     Plate 200 ml onto L-amp plates. Incubate at 37°C overnight.

20

At least 400 cosmid clones were screened for activity against western corn rootworm as described in Example 3. DNA from 5 active clones and 5 non-active clones were used in Southern hybridizations. Results demonstrated that hybridization using the above described oligonucleotide probe correlated with western corn rootworm activity (Table 18).

Cosmid clones P3-12 and P5-4 have been deposited with the Agricultural Research Service Patent Culture Collecton (NRRL) and given accession nos. B-21061 and B-21059 respectively.

5

**Table 18**

**Activity of AB78 cosmid clones against western corn rootworm.**

10

Clone	Mean percent mortality (N=4)
-------	---------------------------------

Clones which hybridize with probe

15

P1-73	47
P1-83	64
P2-2	69
P3-12	85
P5-4	97

20

Clones which do not hybridize with probe

25

P1-2	5
P3-8	4
P3-9	12
P3-18	0
P4-6	9

**Example 10. Identification of a 6 kb region active against western corn rootworm.**

DNA from P3-12 was partially digested with restriction enzyme *Sau* 3A, and ligated into the *E. coli* vector pUC19 and transformed into *E. coli*. A DNA probe specific for the 80 kDa protein was synthesized by PCR amplification of a portion of P3-12 DNA. The oligonucleotides MK113 and MK117, which hybridize to portions of VIP-1, were synthesized using the partial amino acid sequence of the 80 kDa protein. Plasmid subclones were identified by colony hybridization to the PCR probe, and tested for activity against western corn rootworm. One such clone, PL2, hybridizes to the PCR fragment, and is active against western corn rootworm by the assay previously described.

A 6 kb *Cla* I restriction fragment from PL2 was cloned into the *Sma* I site of the *E.coli-Bacillus* shuttle vector pHT 3101 (Lereclus, D. *et al*, 1989, FEMS Microbiology Letters 60:211-218) to yield pCIB6201. This construct confers anti-western corn rootworm activity upon both *Bacillus* and *E.coli* strains, in either orientation. pCIB6022 contains this same 6 kb *Cla* I fragment in pBluescript SK(+) (Stratagene), produces equivalent VIP-1 protein (by western blot), and is also active against western corn rootworm.

The nucleotide sequence of pCIP6022 was determined by the dideoxy termination method of Sanger *et al.*, Proc. Natl. Acad. Sci. USA, 74:5463-5467 (1977), using PRISM Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kits and PRISM Sequenase® Terminator Double-Stranded DNA Sequencing Kit and analyzed on AB1 373 automatic sequencer. The sequence is given in SEQ ID NO:1. pCIB6022 was deposited with the Agricultural Research Service, Patent Culture Collection, (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, USA, and given the Accession No. NRRL B-21222.

**Example 11. Functional dissection of the VIP-1 DNA region.**

To confirm that the VIP-1 open reading frame (ORF) is necessary for insecticidal activity a translational frameshift mutation was created in the gene. The restriction enzyme Bgl II  
5 recognizes a unique site located 1758 bp into the coding region of VIP-1. pCIB6201 was digested with Bgl II, and the single-stranded ends filled-in with DNA polymerase (Klenow fragment) and dNTPS. The plasmid was re-ligated and transformed into E. coli. The resulting plasmid, pCIB6203, contains a four nucleotide insertion in the coding region of VIP-1. pCIB6203 does not confer insecticidal activity, confirming that VIP-1 is an essential component  
10 of western corn rootworm activity.

To further define the region necessary to encode VIP-1, subclones of the VIP-1 and VIP-2 (auxiliary protein) region were constructed and tested for their ability to complement the mutation in pCIB6203. pCIB6023 contains the 3.7kb Xba I-EcoRV fragment in pBluescript SK(+) (Stratagene). Western blot analysis indicates that pCIB6023 produces VIP-1 protein of  
15 equal size and quantity as clones PL2 and pCIB 6022. pCIB6023 contains the entire gene for the 80kd protein. pCIB6023 was deposited with the Agricultural Research Service, Patent Culture Collection, (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, USA, and given the Accession No. NRRL B-21223.

pCIB6023 shows some western corn rootworm activity. However, the level of activity is  
20 less than the activity of pCIB6022. A mixture of cells containing pCIB6203 (VIP-1-mutated, and VIP-2) and cells containing pCIB6023(only VIP-1) shows high activity against western corn rootworm. Thus, pCIB6023 must produce functional VIP-1 gene product, and pCIB6203 must produce a functional VIP-2 gene product. These results suggest a requirement for additional gene product(s) from the VIP-2 region, in combination with VIP-1, to confer maximal western  
25 corn rootworm activity. See Table 19.

**TABLE 19**  
**Characterization of pCIB 6022**

5

	<u>Construct(s)</u> <u>tested</u>	<u>Activity</u> <u>vs WCRV</u>
	pCIB6022	+++
	pCIB6023	+
	pCIB6203	-
	+ pCIB6203 pCIB6023	+++

10 Boxed regions represent the extent of VIP-1. Light shading indicates the regions encoding the 80 kDa peptide observed in *Bacillus*. Dark shading represents the N-terminal amino acids predicted by the DNA sequence of VIP-1. Large "X" represents the location of the frameshift mutation introduced into VIP-1. Arrows represent constructs transcribed by the beta-galactosidase promoter. Restriction sites: C - *Cla* I; X - *Xba* I; S - *Sca* I; RI - *Eco* RI; B - *Bgl* II; RV - *Eco* RV.

**Example 12. AB78 Antibody Production**

Antibody production was initiated in 2 Lewis rats to allow for both the possibility of moving to production of hybridoma cell lines and also to produce enough serum for limited screening of cDNA library. Another factor was the very limited amount of antigen available and the fact that it could only be produced to purity by PAGE and subsequent electrotransfer to nitrocellulose.

Due to the limited availability of antigen on nitrocellulose, the nitrocellulose was emulsified in DMSO and injected into the hind footpads of the animals to elicit B-cell production in the popliteal lymph nodes just upstream. A strong reacting serum was produced by western analysis within the first production bleed. Several subsequent injections and bleeds produced enough serum to accomplish all of the screening required.

Hybridoma production with one of the rats was then initiated. The popliteal lymph node was excised, macerated, and the resulting cells fused with mouse myeloma P3x63Ag8.653. Subsequent cell screening was accomplished as described below. Four initial wells were selected which gave the highest emulsified antigen reaction to be moved to limited dilution cloning. An additional 10 wells were chosen for expansion and cryopreservation.

**Procedure to Emulsify AB78 on nitrocellulose in DMSO for ELISA screening:**

After electrotransfer of AB78 samples run on PAGE to nitrocellulose, the reversible stain Ponceaus is used to visualize all protein transferred. The band corresponding to AB78 toxin, previously identified and N-terminal sequenced, is identified and excised from nitrocellulose. Each band is approximately 1mmx5mm in size to minimize the amount of nitrocellulose emulsified. A single band is placed in a microfuge tube with 250ul of DMSO and macerated using a plastic pestle (Kontes, Vineland, NJ). To aid in emulsification, the DMSO mixture is heated for 2-3 minutes at 37°C-45°C. Some further maceration might be necessary following heating; however, all of the nitrocellulose should be emulsified. Once the AB78 is

emulsified, the sample is placed on ice. In preparation for microtiter plate coating with the emulsified antigen, the sample must be diluted in borate buffered saline as follows: 1:5, 1:10, 1:15, 1:20, 1:30, 1:50, 1:100, and 0. The coating antigen must be prepared fresh immediately prior to use.

5           ELISA protocol:

1. Coat with AB78/DMSO in BBS. Incubate overnight at 4°C.
2. Wash plate 3X with 1X ELISA wash buffer.
3. Block (1% BSA & 0.05% Tween 20 in PBS) for 30 minutes at Room Temperature.
- 10       4. Wash plate 3X with 1X ELISA wash buffer.
5. Add Rat Serum. Incubate 1.5 hours at 37°C.
6. Wash plate 3X with 1X ELISA wash buffer.
7. Add Goat anti-Rat at a conc. of 2ug/ml in ELISA diluent. Incubate 1 hr. at 37°C.
8. Wash plate 3X with 1X ELISA wash buffer.
- 15       9. Add Rabbit anti-Goat Alkaline Phosphatase at 2ug/ml in ELISA diluent. Incubate 1Hr. at 37°C.
10. Wash 3X with 1X ELISA wash buffer.
11. Add Substrate. Incubate 30 minutes at Room Temperature.
- 20       12. Stop with 3N NaOH after 30 minutes.



**Example 13. Activation of insecticidal activity of non-active Bt strains with AB78 VIP clones.**

Adding pCIB6203 together with culture supernatant from a Bt strain GC91 produces 100% mortality in Diabrotica virgifera virgifera. Neither pCIB6203 nor GC91 is active on

5 Diabrotica virgifera virgifera by itself. Data are shown below:

Test material	Percent <u>Diabrotica</u> mortality
pCIB6203	0
GC91	16
pCIB6203 + GC91	100
Control	0

**Example 14. Isolation and Biological Activity of B.cereus AB81.**

10

A second B. cereus strain, designated AB81, was isolated from grain bin dust samples by standard methodologies. A subculture of AB81 was grown and prepared for bioassay as described in Example 2. Biological activity was evaluated as described in Example 3. The results are as follows:

15

Insect species tested	Percent Mortality
<u>Ostrinia nubilalis</u>	0
<u>Agrotis ipsilon</u>	0
<u>Diabrotica virgifera virgifera</u>	55

20

25

**Example 15. Isolation and Biological Activity of *B. thuringiensis* AB6.**

A *B. thuringiensis* strain, designated AB6, was isolated from grain bin dust samples by standard methods known in the art. A subculture of AB6 was grown and prepared for bioassay as described in Example 2. Half of the sample was autoclaved 15 minutes to test for the presence of  $\beta$ -exotoxin.

Biological activity was evaluated as described in Example 3. The results are as follows:

Insect species tested	Percent Mortality
<i>Ostrinia nubilalis</i>	0
<i>Agrotis ipsilon</i>	100
<i>Agrotis ipsilon</i> (autoclaved sample)	0
<i>Diabrotica virgifera virgifera</i>	0

Strain AB6 has been deposited in the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, USA, and given Accession No. NRRL B-21060.

**Example 16. Isolation and Biological characterization of *B. thuringiensis* AB88.**

A Bt strain, designated AB88, was isolated from grain bin dust samples by standard methodologies. A subculture of AB88 was grown and prepared for bioassay as described in Example 2. Half of the sample was autoclaved 15 minutes to test for the presence of  $\beta$ -exotoxin. Biological activity was evaluated against a number of insect species as described in Example 3. The results are as follows:

Insect species tested	Order	Percent mortality of culture supernatant	
		Non-autoclaved	Autoclaved
<u>Agrotis ipsilon</u>	Lepidoptera	100	5
<u>Ostrinia nubilalis</u>	Lepidoptera	100	0
<u>Spodoptera</u>			
<u>frugiperda</u>	Lepidoptera	100	4
<u>Helicoverpa zea</u>	Lepidoptera	100	12
<u>Heliothis virescens</u>	Lepidoptera	100	12
<u>Leptinotarsa</u>			
<u>decemlineata</u>	Coleoptera	0	0
<u>Diabrotica virgifera</u>			
<u>virgifera</u>	Coleoptera	0	5

Delta-endotoxin crystals were purified from strain AB88 by standard methodologies. No activity from pure crystals was observed when bioassayed against Agrotis ipsilon.

**5 Example 17. Purification of VIPs from Strain AB88:**

Bacterial liquid culture was grown overnight at 30°C in TB media. Cells were spun out and the supernatant kept. Proteins were precipitated with ammonium sulfate (70% saturation), centrifuged and the pellet kept. The pellet was resuspended in the original volume of 20 mM Tris pH 7.5 and dialyzed against the same buffer. AB88 dialysate was more turbid than  
 10 comparable material from AB78. AB88 proteins have been separated by several different methods following clarification including isoelectric focusing (Rotofor, BioRad, Hercules, CA), precipitation at pH 4.5, ion-exchange chromatography, size exclusion chromatography and ultrafiltration.

European Corn Borer-active protein remained in the pellet obtained by pH 4.5  
 15 precipitation of dialysate. When preparative IEF was done on the dialysate using pH 3-10 ampholytes, ECB insecticidal activity was found in all fractions with pH of 7 or greater. SDS-PAGE of these fractions showed protein bands of MW ~60 kDa and ~80 kDa. The 60 kDa and 80 kDa bands were separated by anion exchange HPLC on a Poros-Q column (PerSeptive

Biosystems, Cambridge, MA). N-terminal sequence was obtained from two fractions containing proteins of slightly differing MW, but both of approximately 60 kDa in size. The sequences obtained were similar to each other and to some  $\delta$ -endotoxins.

anion exchange fraction 23 (smaller): xEPFVSAxxxQxxx (SEQ ID NO:10)

5 anion exchange fraction 28 (larger): xEYENVEPFVSAx (SEQ ID NO:11)

When the (active) pH 4.5 pellet was further separated by anion exchange on a Poros-Q column, activity was found only in fractions with a major band of ~60 kDa.

Black Cutworm-active protein also remained in the pellet when AB88 dialysate was brought down to pH 4.5. In preparative IEF using pH 3-10 ampholytes, activity was not found  
10 in the ECB-active IEF fractions; instead, it was highest in a fraction of pH 4.5-5.0. Its major components have molecular weights of ~35 and ~80 kDa.

The pH 4.5 pellet was separated by anion exchange HPLC to yield fractions containing only the 35 kDa material and fractions containing both 35 kDa and 80 kDa bands.

#### 15 **Example 18. Characterization of AB88 VIP.**

Fractions containing the various lepidopteran active vegetative proteins were generated as described in Example 17. Analysis of active fractions demonstrates that different VIP's are responsible for the different lepidopteran species activity.

20

The Agrotis ipsilon activity is due to an 80 kDa and or a 35 kDa protein either delivered singly or in combination. These proteins are not related to any  $\delta$ -endotoxins from Bt as evidenced by the lack of sequence homology of known Bt  $\delta$ -endotoxin sequences. Also, these proteins are not found in the AB88  $\delta$ -endotoxin crystal. N-terminal sequences of the major  $\delta$ -

endotoxin proteins were compared with the N-terminal sequences of the 80 kDa and 35 kDa VIP and reveal no sequence homology. A summary of the results follows:

<u>Agrotis</u> VIP N-terminal sequences	N-terminal sequence of major $\delta$ -endotoxin proteins
	130 kDa MDNNPNINE (SEQ ID NO:14)
80 kDa MNKNNTKLPTRALP (SEQ ID NO:12)	80 kDa MDNNPNINE (SEQ ID NO:15)
35 kDa ALSENTGKDGGYTVP (SEQ ID NO:13)	60 kDa MNVLNSGRTTI (SEQ ID NO:16)

- 5 The Ostrinia nubilalis activity is due to a 60 kDa VIP and the Spodoptera frugiperda activity is due to a VIP of unknown size.

Bacillus thuringiensis strain AB88 has been deposited in the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University  
10 Street, Peoria, Illinois 61604, USA and given the Accession No. NRRL B-21225.

**Example 19. Isolation and Biological Activity of Other Bacillus sp.**

Other Bacillus species have been isolated which produce proteins with insecticidal  
15 activity during vegetative growth. These strains were isolated from environmental samples by standard methodologies. Isolates were prepared for bioassay and assayed as described in Examples 2 and 3 respectively. Isolates which produced insecticidal proteins during vegetative growth with activity against Agrotis ipsilon in the bioassay are tabulated below.

<u>Bacillus isolate</u>	Presence of $\delta$ -endotoxin crystal	Percent mortality
AB6	+	100
AB53	-	80
AB88	+	100
AB195	-	60
AB211	-	70
AB217	-	83
AB272	-	80
AB279	-	70
AB289	+	100
AB292	+	80
AB294	-	100
AB300	-	80
AB359	-	100

Isolates AB289, AB294 and AB359 have been deposited in the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria IL 61604, USA and given the Accession Numbers NRRL B-21227, NRRL B-21229, and NRRL B-21226 respectively.

Bacillus isolates which produce insecticidal proteins during vegetative growth with activity against Diabrotica virgifera virgifera are tabulated below.

10

<u>Bacillus isolate</u>	Presence of $\delta$ -endotoxin crystal	Percent mortality
AB52	-	50
AB59	-	71
AB68	+	60
AB78	-	100
AB122	-	57
AB218	-	64
AB256	-	64

Isolates AB59 and AB256 have been deposited in the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria Illinois 61604, USA, and given the Accession Numbers NRRL B-21228 and B-21230, respectively.

5

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated  
10 by reference.

The following deposits have been made at Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A.:

- |    |   |                            |
|----|---|----------------------------|
| 15 | 1. <u>E. coli</u> PL2                       | Accession No. NRRL B-21221 |
|    | 2. <u>E. coli</u> pCIB 6022                 | Accession No. NRRL B-21222 |
|    | 3. <u>E. coli</u> pCIB 6023                 | Accession No. NRRL B-21223 |
|    | 4. <u>Bacillus thuringiensis</u> HD73-78VIP | Accession No. NRRL B-21224 |
|    | 5. <u>Bacillus thuringiensis</u> AB88       | Accession No. NRRL B-21225 |
| 20 | 6. <u>Bacillus thuringiensis</u> AB359      | Accession No. NRRL B-21226 |
|    | 7. <u>Bacillus thuringiensis</u> AB289      | Accession No. NRRL B-21227 |
|    | 8. <u>Bacillus</u> sp. AB59                 | Accession No. NRRL B-21228 |
|    | 9. <u>Bacillus</u> sp. AB294                | Accession No. NRRL B-21229 |
|    | 10. <u>Bacillus</u> sp. AB256               | Accession No. NRRL B-21230 |
| 25 | 11. <u>E. coli</u> P5-4                     | Accession No. NRRL B-21059 |

- |                                       |                            |
|---------------------------------------|----------------------------|
| 12. <u>E. coli</u> P3-12              | Accession No. NRRL B-21061 |
| 13. <u>Bacillus cereus</u> AB78       | Accession No. NRRL B-21058 |
| 14. <u>Bacillus thuringiensis</u> AB6 | Accession No. NRRL B-21060 |

5

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.



## SEQUENCE LISTING

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(ii) TITLE OF INVENTION: Novel Pesticidal Proteins and Strains

(iii) NUMBER OF SEQUENCES: 18

(iv) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: US 08/037,057  
(B) FILING DATE: 25-MAR-1993

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 6106 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Bacillus cereus  
(B) STRAIN: AB78  
(C) INDIVIDUAL ISOLATE: NRRL B-21058

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1082..1810  
(D) OTHER INFORMATION: /product= "VIP-2"  
/label= ORF-1

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1925..2470  
(D) OTHER INFORMATION: /product= "VIP-2"  
/label= ORF-2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATCGATACAA TGTTGTTT CTTAGACCGG TAGTCTCTGT AATTTGTTTA ATGCTATATT	60
CTTTACTTTG ATACATTTTA ATAGCCATTT CAACCTTATC AGTATGTTT TGTGGTCTTC	120
CTCCTTTTTT TCCACGAGCT CTAGCTGCGT TTAATCCTGT TTTGGTACGT TCGCTAATAA	180
TATCTCTTTC TAATTCTGCA ATACTTGCCA TCATTGAAA GAAGAATTTC CCCATAGCAT	240
TAGAGGTATC AATGTTGTCA TGAATAGAAA TAAAATCTAC ACCTAGCTCT TTGAATTTTT	300
CACTTAACCTC AATTAGGTGT TTTGTAGAGC GAGAAATTCG ATCAAGTTTG TAAACAACATA	360

TCTTATCGCC TTTACGTAAT ACTTTTAGCA ACTCTTCGAG TTGAGGGCGC TCTTTTTTTA	420
TTCTGTAT TTTCTCCTGA TATAGCCTTT CTACACCATA TTGTTGCAAA GCATCTATTT	480
GCATATCGAG ATTTTGTTC TCTGTGCTGA CACGAGCATA ACCAAAAATC AAATTGGTTT	540
CACITCCTAT CTAAATATAT CTATTAAAAT AGCACCAAAA ACCTTATTAA ATTAAAATAA	600
GGAACCTTGT TTTTGGATAT GGATTTTGGT ACTCAATATG GATGAGTTTT TAACGCTTTT	660
GTTAAAAAAC AAACAAGTGC CATAAACGGT CGTTTTTGGG ATGACATAAT AAATAATCTG	720
TTTGATTAAC CTAACCTTGT ATCCTTACAG CCCAGTTTTA TTTGTACTTC AACTGACTGA	780
ATATGAAAAC AACATGAAGG TTTCATAAAA TTTATATATT TTCCATAACG GATGCTCTAT	840
CTTTAGGTTA TAGTTAAATT ATAAGAAAAA AACAAACGGA GGGAGTGAAA AAAAGCATCT	900
TCTCTATAAT TTTACAGGCT CTTTAATAAG AAGGGGGGAG ATTAGATAAT AAATATGAAT	960
ATCTATCTAT AATTGTTTGC TTCTACAATA ACTTATCTAA CTTTCATATA CAACAACAAA	1020
ACAGACTAAA TCCAGATTGT ATATTCAATTT TCAGTTGTTC CTTTATAAAA TAATTCATA	1080
A ATG AAA AGA ATG GAG GGA AAG TTG TTT ATG GTG TCA AAA AAA TTA Met Lys Arg Met Glu Gly Lys Leu Phe Met Val Ser Lys Lys Leu	1126
1 5 10 15	
CAA GTA GTT ACT AAA ACT GTA TTG CTT AGT ACA GTT TTC TCT ATA TCT Gln Val Val Thr Lys Thr Val Leu Leu Ser Thr Val Phe Ser Ile Ser	1174
20 25 30	
TTA TTA AAT AAT GAA GTG ATA AAA GCT GAA CAA TTA AAT ATA AAT TCT Leu Leu Asn Asn Glu Val Ile Lys Ala Glu Gln Leu Asn Ile Asn Ser	1222
35 40 45	
CAA AGT AAA TAT ACT AAC TTG CAA AAT CTA AAA ATC ACT GAC AAG GTA Gln Ser Lys Tyr Thr Asn Leu Gln Asn Leu Lys Ile Thr Asp Lys Val	1270
50 55 60	
GAG GAT TTT AAA GAA GAT AAG GAA AAA GCG AAA GAA TGG GGG AAA GAA Glu Asp Phe Lys Glu Asp Lys Glu Lys Ala Lys Glu Trp Gly Lys Glu	1318
65 70 75	
AAA GAA AAA GAG TGG AAA CTA ACT GCT ACT GAA AAA GGA AAA ATG AAT Lys Glu Lys Glu Trp Lys Leu Thr Ala Thr Glu Lys Gly Lys Met Asn	1366
80 85 90 95	
AAT TTT TTA GAT AAT AAA AAT GAT ATA NAG ACA AAT TAT AAA GAA ATT Asn Phe Leu Asp Asn Lys Asn Asp Ile Xaa Thr Asn Tyr Lys Glu Ile	1414
100 105 110	
ACT TTT TCT ATG GCA GGC TCA TTT GAA GAT GAA ATA AAA GAT TTA AAA Thr Phe Ser Met Ala Gly Ser Phe Glu Asp Glu Ile Lys Asp Leu Lys	1462
115 120 125	
GAA ATT GAT AAG ATG TTT GAT AAA ACC AAT CTA TCA AAT TCT ATT ATC Glu Ile Asp Lys Met Phe Asp Lys Thr Asn Leu Ser Asn Ser Ile Ile	1510
130 135 140	

ACC TAT AAA AAT GTG GAA CCG ACA ACA ATT GGA TTT AAT AAA TCT TTA Thr Tyr Lys Asn Val Glu Pro Thr Thr Ile Gly Phe Asn Lys Ser Leu 145 150 155	1558
ACA GAA GGT AAT ACG ATT AAT TCT GAT GCA ATG GCA CAG TTT AAA GAA Thr Glu Gly Asn Thr Ile Asn Ser Asp Ala Met Ala Gln Phe Lys Glu 160 165 170 175	1606
CAA TTT TTA GAT AGG GAT ATT AAG TTT GAT AGT TAT CTA GAT ACG CAT Gln Phe Leu Asp Arg Asp Ile Lys Phe Asp Ser Tyr Leu Asp Thr His 180 185 190	1654
TTA ACT GCT CAA CAA GTT TCC AGT AAA GAA AGA GTT ATT TTG AAG GTT Leu Thr Ala Gln Gln Val Ser Ser Lys Glu Arg Val Ile Leu Lys Val 195 200 205	1702
ACG GTT CCG AGT GGG AAA GGT TCT ACT ACT CCA ACA AAA GCA GGT GTC Thr Val Pro Ser Gly Lys Gly Ser Thr Thr Pro Thr Lys Ala Gly Val 210 215 220	1750
ATT TTA AAT AAT AGT GAA TAC AAA ATG CTC ATT GAT AAT GGG TAT ATG Ile Leu Asn Asn Ser Glu Tyr Lys Met Leu Ile Asp Asn Gly Tyr Met 225 230 235	1798
GTC CAT GTA GAT TAAGGTATCA AAAGTGGTGA AAAAAGGGGG TGGAGTGCCT Val His Val Asp 240	1850
TACAAATTGA AGGGACTTTA AAAAAGAGTC TTGACTTTAA AAATGATATA AATGCTGAAG	1910
CGCATAGCTG GGGT ATG AAG AAT TAT GAA GAG TGG GCT AAA GAT TTA ACC Met Lys Asn Tyr Glu Glu Trp Ala Lys Asp Leu Thr 1 5 10	1960
GAT TCG CAA AGG GAA GCT TTA GAT GGG TAT GCT AGG CAA GAT TAT AAA Asp Ser Gln Arg Glu Ala Leu Asp Gly Tyr Ala Arg Gln Asp Tyr Lys 15 20 25	2008
GAA ATC AAT AAT TAT TTA AGA AAT CAA GGC GGA AGT GGA AAT GAA AAA Glu Ile Asn Asn Tyr Leu Arg Asn Gln Gly Gly Ser Gly Asn Glu Lys 30 35 40	2056
CTA GAT GCT CAA ATA AAA AAT ATT TCT GAT GCT TTA GGG AAG AAA CCA Leu Asp Ala Gln Ile Lys Asn Ile Ser Asp Ala Leu Gly Lys Lys Pro 45 50 55 60	2104
ATA CCG GAA AAT ATT ACT GTG TAT AGA TGG TGT GGC ATG CCG GAA TTT Ile Pro Glu Asn Ile Thr Val Tyr Arg Trp Cys Gly Met Pro Glu Phe 65 70 75	2152
GGT TAT CAA ATT AGT GAT CCG TTA CCT TCT TTA AAA GAT TTT GAA GAA Gly Tyr Gln Ile Ser Asp Pro Leu Pro Ser Leu Lys Asp Phe Glu Glu 80 85 90	2200
CAA TTT TTA AAT ACA ATC AAA GAA GAC AAA GGA TAT ATG AGT ACA AGC Gln Phe Leu Asn Thr Ile Lys Glu Asp Lys Gly Tyr Met Ser Thr Ser 95 100 105	2248
TTA TCG AGT GAA CGT CTT GCA GCT TTT GGA TCT AGA AAA ATT ATA TTA	2296

Leu Ser Ser Glu Arg Leu Ala Ala Phe Gly Ser Arg Lys Ile Ile Leu 110 115 120	
CGA TTA CAA GTT CCG AAA GGA AGT ACG GGT GCG TAT TTA AGT GCC ATT Arg Leu Gln Val Pro Lys Gly Ser Thr Gly Ala Tyr Leu Ser Ala Ile 125 130 135 140	2344
GGT GGA TTT GCA AGT GAA AAA GAG ATC CTA CTT GAT AAA GAT AGT AAA Gly Gly Phe Ala Ser Glu Lys Glu Ile Leu Leu Asp Lys Asp Ser Lys 145 150 155	2392
TAT CAT ATT GAT AAA GTA ACA GAG GTA ATT ATT AAA GGT GTT AAG CGA Tyr His Ile Asp Lys Val Thr Glu Val Ile Ile Lys Gly Val Lys Arg 160 165 170	2440
TAT GTA GTG GAT GCA ACA TTA TTA ACA AAT TAAGGAGATG AAAAATATGA Tyr Val Val Asp Ala Thr Leu Thr Asn 175 180	2490
AGAAAAAGTT AGCAAGTGTT GTAACGTGTA CGTTATTAGC TCCTATGTTT TTGAATGGAA	2550
ATGTGAATGC TGTTTACGCA GACAGCAAAA CAAATCAAAT TTCTACAACA CAGAAAAATC	2610
AACAGAAAGA GATGGACCGA AAAGGATTAC TTGGGTATTA TTCAAAGGA AAAGATTTTA	2670
GTAATCTTAC TATGTTTGCA CCGACACGTG ATAGTACTCT TATTTATGAT CAACAAACAG	2730
CAAATAAACT ATTAGATAAA AAACAACAAG AATATCAGTC TATTCGTTGG ATTGGTTTGA	2790
TTCAGAGTAA AGAAACGGGA GATTTACAT TTAACCTTATC TGAGGATGAA CAGGCAATTA	2850
TAGAAATCAA TGGGAAAATT ATTTCTAATA AAGGGAAAGA AAAGCAAGTT GTCCATTTAG	2910
AAAAAGGAAA ATTAGTTCCA ATCAAAATAG AGTATCAATC AGATACAAA TTTAATATTG	2970
ACAGTAAAC ATTTAAAGAA CTTAAATTAT TTAATAAGA TAGTCAAAAC CAACCCACAGC	3030
AAGTCCAGCA AGATGAACTG AGAAATCCTG AATTTAACAA GAAAGAATCA CAGGAATTCT	3090
TAGCGAAACC ATCGAAAATA AATCTTTTCA CTCAAMAAAT GAAAAGGGAA ATTGATGAAG	3150
ACACGGATAC GGATGGGGAC TCTATTCCTG ACCTTTGGGA AGAAAATGGG TATACGATTC	3210
AMAATAGAAT CGCTGTAAAG TGGGACGATT CTCTAGCAAG TAAAGGGTAT ACGAAATTTG	3270
TTTCAAATCC ACTAGAAAGT CACACAGTTG GTGATCCTTA TACAGATTAT GAAAAGGCAG	3330
CAAGAGATCT AGATTTGTCA AATGCAAAGG AAACGTTTAA CCCATTGGTA GCTGCTTTTC	3390
CAAGTGTGAA TGTTAGTATG GAAAAGGTGA TATTATCACC AAATGAAAAT TTATCCAATA	3450
GTGTAGAGTC TCATTCATCC ACGAATTGGT CTTATACAAA TACAGAAGGT GCTTCTGTTG	3510
AAGCGGGGAT TGGACCAAAA GGTATTTCTG TCGGAGTTAG CGTAAACTAT CAACACTCTG	3570
AAACAGTTGC ACAAGAATGG GGAACATCTA CAGGAAATAC TTCGCAATTC AATACGGCTT	3630
CAGCGGGATA TTAAATGCA AATGTTCGAT ATAACAATGT AGGAACTGGT GCCATCTACG	3690
ATGTAAACC TACAACAAGT TTTGTATTAA ATAACGATAC TATCGCAACT ATTACGGCGA	3750

AATCTAATTC TACAGCCTTA AATATATCTC CTGGAGAAAG TTACCCGAAA AAAGGACAAA	3810
ATGGAATCGC AATAACATCA ATGGATGATT TTAATTCCCA TCCGATTACA TTAAATAAAA	3870
AACAAGTAGA TAATCTGCTA AATAATAAAC CTATGATGTT GGAAACAAAC CAAACAGATG	3930
GTGTTTATAA GATAAAAGAT ACACATGGAA ATATAGTAAC TGGCGGAGAA TGAATGGTG	3990
TCATACAACA AATCAAGGCT AAAACAGCGT CTATTATTGT GGATGATGGG GAACGTGTAG	4050
CAGAAAAACG TGTAGCGGCA AAAGATTATG AAAATCCAGA AGATAAAACA CCGTCTTTAA	4110
CTTTAAAAGA TGCCCTGAAG CTTTCATATC CAGATGAAAT AAAAGAAATA GAGGGATTAT	4170
TATATTATAA AAACAAACCG ATATACGAAT CGAGCGTTAT GACTTACTTA GATGAAAATA	4230
CAGCAAAAGA AGTGACCAA CAATTAAATG ATACCACTGG GAAATTTAAA GATGTAAGTC	4290
ATTTATATGA TGTAAACTG ACTCCAAAAA TGAATGTTAC AATCAAATTG TCTATACTTT	4350
ATGATAATGC TGAGTCTAAT GATAACTCAA TTGGTAAATG GACAAACACA AATATTGTTT	4410
CAGGTGGAAA TAACGGAAAA AAACAATATT CTTCTAATAA TCCGGATGCT AATTTGACAT	4470
TAAATACAGA TGCTCAAGAA AAATTAAATA AAAATCGTGA CTATTATATA AGTTTATATA	4530
TGAAGTCAGA AAAAAACACA CAATGTGAGA TTACTATAGA TGGGGAGATT TATCCGATCA	4590
CTACAAAAAC AGTGAATGTG AATAAAGACA ATTACAAAAG ATTAGATATT ATAGCTCATA	4650
ATATAAAAAG TAATCCAATT TCTTCACTTC ATATTAAAAC GAATGATGAA ATAACTTTAT	4710
TTTGGGATGA TATTTCTATA ACAGATGTAG CATCAATAAA ACCGGAAAAT TTAACAGATT	4770
CAGAAATTAA ACAGATTTAT AGTAGGTATG GTATTAAGTT AGAAGATGGA ATCCTTATTG	4830
ATAAAAAAGG TGGGATTCAT TATGGTGAAT TTATTAATGA AGCTAGTTT AATATTGAAC	4890
CATTGCCAAA TTATGTGACC AAATATGAAG TTACTTATAG TAGTGAGTTA GGACCAAACG	4950
TGAGTGACAC ACTTGAAAGT GATAAAATTT ACAAGGATGG GACAATTAAA TTTGATTTTA	5010
CCAAATATAG TAAAAATGAA CAAGGATTAT TTTATGACAG TGGATTAAAT TGGGACTTTA	5070
AAATTAATGC TATTACTTAT GATGGTAAAG AGATGAATGT TTTTCATAGA TATAATAAAT	5130
AGTTATTATA TCTATGAAGC TGGTGCTAAA GATAGTGTA AAGTTAATAT ACTGTAGGAT	5190
TGTAATAAAA GTAATGGAAT TGATATCGTA CTTTGGAGTG GGGGATACTT TGTAATAGT	5250
TCTATCAGAA ACATTAGACT AAGAAAAGTT ACTACCCCA CTTGAAAATG AAGATTCAAC	5310
TGATTACAAA CAACCTGTTA AATATTATAA GGTTTAAACA AAATATTAAA CTCTTTATGT	5370
TAATACTGTA ATATAAGAG TTTAATTGTA TTCAAATGAA GCTTTCCAC AAAATTAGAC	5430
TGATTATCTA ATGAAATAAT CAGTCTAATT TTGTAGAACA GGTCTGGTAT TATTGTACGT	5490
GGTCACTAAA AGATATCTAA TATTATTGGG CAAGCGGTT CATGATTGAA TCCTCGAATG	5550

TCTTGCCCTT TTCATTTATT TAAGAAGGAT TGTGGAGAAA TTATGGTTTA GATAATGAAG	5610
AAAGACTTCA CTCTAATTT TTGATGTTAA ATAAATCAAA ATTTGGCGAT TCACATTGTT	5670
TAATCCACTG ATAAAACATA CTGGAGTGTT CTAAAAAAT CAGCTTTTTT CTTTATAAAA	5730
TTTTGCTTAG CGTACGAAAT TCGTGTTTTG TTGGTGGGAC CCCATGCCCA TCAACTTAAG	5790
AGTAAATTAG TAATGAACTT TCGTTCATCT GGATTAAAAT AACCTCAAAT TAGGACATGT	5850
TTTTAAAAAT AAGCAGACCA AATAAGCCTA GAATAGGTAT CATTTTTTAAA AATTATGCTG	5910
CTTCTTTTG TTTTCCAAAT CCATTATACT CATAAGCAAC ACCCATAATG TCAAAGACTG	5970
TTTTTGCTC ATATCGATAA GCTTGATATC GAATTCCTGC AGCCCGGGGG ATCCACTAGT	6030
TCTAGAGCGG CCGCCACCGC GGTGGAGCTC CAGCTTTTGT TCCCTTTAGT GAGGGTTAAG	6090
TTCGAGCTTG TCGTGG	6106

## (2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 243 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met	Lys	Arg	Met	Glu	Gly	Lys	Leu	Phe	Met	Val	Ser	Lys	Lys	Leu	Gln	1	5	10	15
Val	Val	Thr	Lys	Thr	Val	Leu	Leu	Ser	Thr	Val	Phe	Ser	Ile	Ser	Leu	20	25	30	
Leu	Asn	Asn	Glu	Val	Ile	Lys	Ala	Glu	Gln	Leu	Asn	Ile	Asn	Ser	Gln	35	40	45	
Ser	Lys	Tyr	Thr	Asn	Leu	Gln	Asn	Leu	Lys	Ile	Thr	Asp	Lys	Val	Glu	50	55	60	
Asp	Phe	Lys	Glu	Asp	Lys	Glu	Lys	Ala	Lys	Glu	Trp	Gly	Lys	Glu	Lys	65	70	75	80
Glu	Lys	Glu	Trp	Lys	Leu	Thr	Ala	Thr	Glu	Lys	Gly	Lys	Met	Asn	Asn	85	90	95	
Phe	Leu	Asp	Asn	Lys	Asn	Asp	Ile	Xaa	Thr	Asn	Tyr	Lys	Glu	Ile	Thr	100	105	110	
Phe	Ser	Met	Ala	Gly	Ser	Phe	Glu	Asp	Glu	Ile	Lys	Asp	Leu	Lys	Glu	115	120	125	
Ile	Asp	Lys	Met	Phe	Asp	Lys	Thr	Asn	Leu	Ser	Asn	Ser	Ile	Ile	Thr	130	135	140	

Tyr Lys Asn Val Glu Pro Thr Thr Ile Gly Phe Asn Lys Ser Leu Thr  
 145 150 155 160  
 Glu Gly Asn Thr Ile Asn Ser Asp Ala Met Ala Gln Phe Lys Glu Gln  
 165 170 175  
 Phe Leu Asp Arg Asp Ile Lys Phe Asp Ser Tyr Leu Asp Thr His Leu  
 180 185 190  
 Thr Ala Gln Gln Val Ser Ser Lys Glu Arg Val Ile Leu Lys Val Thr  
 195 200 205  
 Val Pro Ser Gly Lys Gly Ser Thr Thr Pro Thr Lys Ala Gly Val Ile  
 210 215 220  
 Leu Asn Asn Ser Glu Tyr Lys Met Leu Ile Asp Asn Gly Tyr Met Val  
 225 230 235 240  
 His Val Asp

## (2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 182 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Lys Asn Tyr Glu Glu Trp Ala Lys Asp Leu Thr Asp Ser Gln Arg  
 1 5 10 15  
 Glu Ala Leu Asp Gly Tyr Ala Arg Gln Asp Tyr Lys Glu Ile Asn Asn  
 20 25 30  
 Tyr Leu Arg Asn Gln Gly Gly Ser Gly Asn Glu Lys Leu Asp Ala Gln  
 35 40 45  
 Ile Lys Asn Ile Ser Asp Ala Leu Gly Lys Lys Pro Ile Pro Glu Asn  
 50 55 60  
 Ile Thr Val Tyr Arg Trp Cys Gly Met Pro Glu Phe Gly Tyr Gln Ile  
 65 70 75 80  
 Ser Asp Pro Leu Pro Ser Leu Lys Asp Phe Glu Glu Gln Phe Leu Asn  
 85 90 95  
 Thr Ile Lys Glu Asp Lys Gly Tyr Met Ser Thr Ser Leu Ser Ser Glu  
 100 105 110  
 Arg Leu Ala Ala Phe Gly Ser Arg Lys Ile Ile Leu Arg Leu Gln Val  
 115 120 125  
 Pro Lys Gly Ser Thr Gly Ala Tyr Leu Ser Ala Ile Gly Gly Phe Ala  
 130 135 140



Ser Glu Lys Glu Ile Leu Leu Asp Lys Asp Ser Lys Tyr His Ile Asp  
 145 150 155 160  
 Lys Val Thr Glu Val Ile Ile Lys Gly Val Lys Arg Tyr Val Val Asp  
 165 170 175  
 Ala Thr Leu Leu Thr Asn  
 180

## (2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2655 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: *Bacillus cereus*
  - (B) STRAIN: AB78
  - (C) INDIVIDUAL ISOLATE: NRRL B-21058
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..2652
  - (C) IDENTIFICATION METHOD: experimental
  - (D) OTHER INFORMATION: /product= "100 kDa protein VIP-1"  
 /evidence= EXPERIMENTAL

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATG AAA AAT ATG AAG AAA AAG TTA GCA AGT GTT GTA ACG TGT ACG TTA	48
Met Lys Asn Met Lys Lys Lys Leu Ala Ser Val Val Thr Cys Thr Leu	
1 5 10 15	
TTA GCT CCT ATG TTT TTG AAT GGA AAT GTG AAT GCT GTT TAC GCA GAC	96
Leu Ala Pro Met Phe Leu Asn Gly Asn Val Asn Ala Val Tyr Ala Asp	
20 25 30	
AGC AAA ACA AAT CAA ATT TCT ACA ACA CAG AAA AAT CAA CAG AAA GAG	144
Ser Lys Thr Asn Gln Ile Ser Thr Thr Gln Lys Asn Gln Gln Lys Glu	
35 40 45	
ATG GAC CGA AAA GGA TTA CTT GGG TAT TAT TTC AAA GGA AAA GAT TTT	192
Met Asp Arg Lys Gly Leu Leu Gly Tyr Tyr Phe Lys Gly Lys Asp Phe	
50 55 60	
AGT AAT CTT ACT ATG TTT GCA CCG ACA CGT GAT AGT ACT CTT ATT TAT	240
Ser Asn Leu Thr Met Phe Ala Pro Thr Arg Asp Ser Thr Leu Ile Tyr	
65 70 75 80	
GAT CAA CAA ACA GCA AAT AAA CTA TTA GAT AAA AAA CAA CAA GAA TAT	288
Asp Gln Gln Thr Ala Asn Lys Leu Leu Asp Lys Lys Gln Gln Glu Tyr	

85	90	95	
CAG TCT ATT CGT TGG ATT GGT TTG ATT CAG AGT AAA GAA ACG GGA GAT Gln Ser Ile Arg Trp Ile Gly Leu Ile Gln Ser Lys Glu Thr Gly Asp 100 105 110			336
TTC ACA TTT AAC TTA TCT GAG GAT GAA CAG GCA ATT ATA GAA ATC AAT Phe Thr Phe Asn Leu Ser Glu Asp Glu Gln Ala Ile Ile Glu Ile Asn 115 120 125			384
GGG AAA ATT ATT TCT AAT AAA GGG AAA GAA AAG CAA GTT GTC CAT TTA Gly Lys Ile Ile Ser Asn Lys Gly Lys Glu Lys Gln Val Val His Leu 130 135 140			432
GAA AAA GGA AAA TTA GTT CCA ATC AAA ATA GAG TAT CAA TCA GAT ACA Glu Lys Gly Lys Leu Val Pro Ile Lys Ile Glu Tyr Gln Ser Asp Thr 145 150 155 160			480
AAA TTT AAT ATT GAC AGT AAA ACA TTT AAA GAA CTT AAA TTA TTT AAA Lys Phe Asn Ile Asp Ser Lys Thr Phe Lys Glu Leu Lys Leu Phe Lys 165 170 175			528
ATA GAT AGT CAA AAC CAA CCC CAG CAA GTC CAG CAA GAT GAA CTG AGA Ile Asp Ser Gln Asn Gln Pro Gln Gln Val Gln Gln Asp Glu Leu Arg 180 185 190			576
AAT CCT GAA TTT AAC AAG AAA GAA TCA CAG GAA TTC TTA GCG AAA CCA Asn Pro Glu Phe Asn Lys Lys Glu Ser Gln Glu Phe Leu Ala Lys Pro 195 200 205			624
TCG AAA ATA AAT CTT TTC ACT CAA MAA ATG AAA AGG GAA ATT GAT GAA Ser Lys Ile Asn Leu Phe Thr Gln Xaa Met Lys Arg Glu Ile Asp Glu 210 215 220			672
GAC ACG GAT ACG GAT GGG GAC TCT ATT CCT GAC CTT TGG GAA GAA AAT Asp Thr Asp Thr Asp Gly Asp Ser Ile Pro Asp Leu Trp Glu Glu Asn 225 230 235 240			720
GGG TAT ACG ATT CAM AAT AGA ATC GCT GTA AAG TGG GAC GAT TCT CTA Gly Tyr Thr Ile Xaa Asn Arg Ile Ala Val Lys Trp Asp Asp Ser Leu 245 250 255			768
GCA AGT AAA GGG TAT ACG AAA TTT GTT TCA AAT CCA CTA GAA AGT CAC Ala Ser Lys Gly Tyr Thr Lys Phe Val Ser Asn Pro Leu Glu Ser His 260 265 270			816
ACA GTT GGT GAT CCT TAT ACA GAT TAT GAA AAG GCA GCA AGA GAT CTA Thr Val Gly Asp Pro Tyr Thr Asp Tyr Glu Lys Ala Ala Arg Asp Leu 275 280 285			864
GAT TTG TCA AAT GCA AAG GAA ACG TTT AAC CCA TTG GTA GCT GCT TTT Asp Leu Ser Asn Ala Lys Glu Thr Phe Asn Pro Leu Val Ala Ala Phe 290 295 300			912
CCA AGT GTG AAT GTT AGT ATG GAA AAG GTG ATA TTA TCA CCA AAT GAA Pro Ser Val Asn Val Ser Met Glu Lys Val Ile Leu Ser Pro Asn Glu 305 310 315 320			960
AAT TTA TCC AAT AGT GTA GAG TCT CAT TCA TCC ACG AAT TGG TCT TAT Asn Leu Ser Asn Ser Val Glu Ser His Ser Ser Thr Asn Trp Ser Tyr			1008

325	330	335	
ACA AAT ACA GAA GGT GCT TCT GTT GAA GCG GGG ATT GGA CCA AAA GGT Thr Asn Thr Glu Gly Ala Ser Val Glu Ala Gly Ile Gly Pro Lys Gly 340 345 350			1056
ATT TCG TTC GGA GTT AGC GTA AAC TAT CAA CAC TCT GAA ACA GTT GCA Ile Ser Phe Gly Val Ser Val Asn Tyr Gln His Ser Glu Thr Val Ala 355 360 365			1104
CAA GAA TGG GGA ACA TCT ACA GGA AAT ACT TCG CAA TTC AAT ACG GCT Gln Glu Trp Gly Thr Ser Thr Gly Asn Thr Ser Gln Phe Asn Thr Ala 370 375 380			1152
TCA GCG GGA TAT TTA AAT GCA AAT GTT CGA TAT AAC AAT GTA GGA ACT Ser Ala Gly Tyr Leu Asn Ala Asn Val Arg Tyr Asn Asn Val Gly Thr 385 390 395 400			1200
GGT GCC ATC TAC GAT GTA AAA CCT ACA ACA AGT TTT GTA TTA AAT AAC Gly Ala Ile Tyr Asp Val Lys Pro Thr Thr Ser Phe Val Leu Asn Asn 405 410 415			1248
GAT ACT ATC GCA ACT ATT ACG GCG AAA TCT AAT TCT ACA GCC TTA AAT Asp Thr Ile Ala Thr Ile Thr Ala Lys Ser Asn Ser Thr Ala Leu Asn 420 425 430			1296
ATA TCT CCT GGA GAA AGT TAC CCG AAA AAA GGA CAA AAT GGA ATC GCA Ile Ser Pro Gly Glu Ser Tyr Pro Lys Lys Gly Gln Asn Gly Ile Ala 435 440 445			1344
ATA ACA TCA ATG GAT GAT TTT AAT TCC CAT CCG ATT ACA TTA AAT AAA Ile Thr Ser Met Asp Asp Phe Asn Ser His Pro Ile Thr Leu Asn Lys 450 455 460			1392
AAA CAA GTA GAT AAT CTG CTA AAT AAT AAA CCT ATG ATG TTG GAA ACA Lys Gln Val Asp Asn Leu Leu Asn Asn Lys Pro Met Met Leu Glu Thr 465 470 475 480			1440
AAC CAA ACA GAT GGT GTT TAT AAG ATA AAA GAT ACA CAT GGA AAT ATA Asn Gln Thr Asp Gly Val Tyr Lys Ile Lys Asp Thr His Gly Asn Ile 485 490 495			1488
GTA ACT GGC GGA GAA TGG AAT GGT GTC ATA CAA CAA ATC AAG GCT AAA Val Thr Gly Gly Glu Trp Asn Gly Val Ile Gln Gln Ile Lys Ala Lys 500 505 510			1536
ACA GCG TCT ATT ATT GTG GAT GAT GGG GAA CGT GTA GCA GAA AAA CGT Thr Ala Ser Ile Ile Val Asp Asp Gly Glu Arg Val Ala Glu Lys Arg 515 520 525			1584
GTA GCG GCA AAA GAT TAT GAA AAT CCA GAA GAT AAA ACA CCG TCT TTA Val Ala Ala Lys Asp Tyr Glu Asn Pro Glu Asp Lys Thr Pro Ser Leu 530 535 540			1632
ACT TTA AAA GAT GCC CTG AAG CTT TCA TAT CCA GAT GAA ATA AAA GAA Thr Leu Lys Asp Ala Leu Lys Leu Ser Tyr Pro Asp Glu Ile Lys Glu 545 550 555 560			1680
ATA GAG GGA TTA TTA TAT TAT AAA AAC AAA CCG ATA TAC GAA TCG AGC Ile Glu Gly Leu Leu Tyr Tyr Lys Asn Lys Pro Ile Tyr Glu Ser Ser			1728

66

	805	810	815	
TAT AGT AGT GAG TTA GGA CCA AAC GTG AGT GAC ACA CTT GAA AGT GAT				2496
Tyr Ser Ser Glu Leu Gly Pro Asn Val Ser Asp Thr Leu Glu Ser Asp				
	820	825	830	
AAA ATT TAC AAG GAT GGG ACA ATT AAA TTT GAT TTT ACC AAA TAT AGT				2544
Lys Ile Tyr Lys Asp Gly Thr Ile Lys Phe Asp Phe Thr Lys Tyr Ser				
	835	840	845	
AAA AAT GAA CAA GGA TTA TTT TAT GAC AGT GGA TTA AAT TGG GAC TTT				2592
Lys Asn Glu Gln Gly Leu Phe Tyr Asp Ser Gly Leu Asn Trp Asp Phe				
	850	855	860	
AAA ATT AAT GCT ATT ACT TAT GAT GGT AAA GAG ATG AAT GTT TTT CAT				2640
Lys Ile Asn Ala Ile Thr Tyr Asp Gly Lys Glu Met Asn Val Phe His				
	865	870	875	880
AGA TAT AAT AAA TAG				2655
Arg Tyr Asn Lys				

## (2) INFORMATION FOR SEQ ID NO: 5:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 884 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met	Lys	Asn	Met	Lys	Lys	Lys	Leu	Ala	Ser	Val	Val	Thr	Cys	Thr	Leu
1				5					10					15	
Leu	Ala	Pro	Met	Phe	Leu	Asn	Gly	Asn	Val	Asn	Ala	Val	Tyr	Ala	Asp
		20					25						30		
Ser	Lys	Thr	Asn	Gln	Ile	Ser	Thr	Thr	Gln	Lys	Asn	Gln	Gln	Lys	Glu
		35					40					45			
Met	Asp	Arg	Lys	Gly	Leu	Leu	Gly	Tyr	Tyr	Phe	Lys	Gly	Lys	Asp	Phe
	50				55						60				
Ser	Asn	Leu	Thr	Met	Phe	Ala	Pro	Thr	Arg	Asp	Ser	Thr	Leu	Ile	Tyr
	65				70				75					80	
Asp	Gln	Gln	Thr	Ala	Asn	Lys	Leu	Leu	Asp	Lys	Lys	Gln	Gln	Glu	Tyr
			85					90						95	
Gln	Ser	Ile	Arg	Trp	Ile	Gly	Leu	Ile	Gln	Ser	Lys	Glu	Thr	Gly	Asp
		100					105						110		
Phe	Thr	Phe	Asn	Leu	Ser	Glu	Asp	Glu	Gln	Ala	Ile	Ile	Glu	Ile	Asn
	115					120					125				
Gly	Lys	Ile	Ile	Ser	Asn	Lys	Gly	Lys	Glu	Lys	Gln	Val	Val	His	Leu
	130					135					140				

Glu Lys Gly Lys Leu Val Pro Ile Lys Ile Glu Tyr Gln Ser Asp Thr  
 145 150 155 160  
 Lys Phe Asn Ile Asp Ser Lys Thr Phe Lys Glu Leu Lys Leu Phe Lys  
 165 170 175  
 Ile Asp Ser Gln Asn Gln Pro Gln Gln Val Gln Gln Asp Glu Leu Arg  
 180 185 190  
 Asn Pro Glu Phe Asn Lys Lys Glu Ser Gln Glu Phe Leu Ala Lys Pro  
 195 200 205  
 Ser Lys Ile Asn Leu Phe Thr Gln Xaa Met Lys Arg Glu Ile Asp Glu  
 210 215 220  
 Asp Thr Asp Thr Asp Gly Asp Ser Ile Pro Asp Leu Trp Glu Glu Asn  
 225 230 235 240  
 Gly Tyr Thr Ile Xaa Asn Arg Ile Ala Val Lys Trp Asp Asp Ser Leu  
 245 250 255  
 Ala Ser Lys Gly Tyr Thr Lys Phe Val Ser Asn Pro Leu Glu Ser His  
 260 265 270  
 Thr Val Gly Asp Pro Tyr Thr Asp Tyr Glu Lys Ala Ala Arg Asp Leu  
 275 280 285  
 Asp Leu Ser Asn Ala Lys Glu Thr Phe Asn Pro Leu Val Ala Ala Phe  
 290 295 300  
 Pro Ser Val Asn Val Ser Met Glu Lys Val Ile Leu Ser Pro Asn Glu  
 305 310 315 320  
 Asn Leu Ser Asn Ser Val Glu Ser His Ser Ser Thr Asn Trp Ser Tyr  
 325 330 335  
 Thr Asn Thr Glu Gly Ala Ser Val Glu Ala Gly Ile Gly Pro Lys Gly  
 340 345 350  
 Ile Ser Phe Gly Val Ser Val Asn Tyr Gln His Ser Glu Thr Val Ala  
 355 360 365  
 Gln Glu Trp Gly Thr Ser Thr Gly Asn Thr Ser Gln Phe Asn Thr Ala  
 370 375 380  
 Ser Ala Gly Tyr Leu Asn Ala Asn Val Arg Tyr Asn Asn Val Gly Thr  
 385 390 395 400  
 Gly Ala Ile Tyr Asp Val Lys Pro Thr Thr Ser Phe Val Leu Asn Asn  
 405 410 415  
 Asp Thr Ile Ala Thr Ile Thr Ala Lys Ser Asn Ser Thr Ala Leu Asn  
 420 425 430  
 Ile Ser Pro Gly Glu Ser Tyr Pro Lys Lys Gly Gln Asn Gly Ile Ala  
 435 440 445  
 Ile Thr Ser Met Asp Asp Phe Asn Ser His Pro Ile Thr Leu Asn Lys  
 450 455 460

Lys Gln Val Asp Asn Leu Leu Asn Asn Lys Pro Met Met Leu Glu Thr  
 465 470 475 480  
 Asn Gln Thr Asp Gly Val Tyr Lys Ile Lys Asp Thr His Gly Asn Ile  
 485 490 495  
 Val Thr Gly Gly Glu Trp Asn Gly Val Ile Gln Gln Ile Lys Ala Lys  
 500 505 510  
 Thr Ala Ser Ile Ile Val Asp Asp Gly Glu Arg Val Ala Glu Lys Arg  
 515 520 525  
 Val Ala Ala Lys Asp Tyr Glu Asn Pro Glu Asp Lys Thr Pro Ser Leu  
 530 535 540  
 Thr Leu Lys Asp Ala Leu Lys Leu Ser Tyr Pro Asp Glu Ile Lys Glu  
 545 550 555 560  
 Ile Glu Gly Leu Leu Tyr Tyr Lys Asn Lys Pro Ile Tyr Glu Ser Ser  
 565 570 575  
 Val Met Thr Tyr Leu Asp Glu Asn Thr Ala Lys Glu Val Thr Lys Gln  
 580 585 590  
 Leu Asn Asp Thr Thr Gly Lys Phe Lys Asp Val Ser His Leu Tyr Asp  
 595 600 605  
 Val Lys Leu Thr Pro Lys Met Asn Val Thr Ile Lys Leu Ser Ile Leu  
 610 615 620  
 Tyr Asp Asn Ala Glu Ser Asn Asp Asn Ser Ile Gly Lys Trp Thr Asn  
 625 630 635 640  
 Thr Asn Ile Val Ser Gly Gly Asn Asn Gly Lys Lys Gln Tyr Ser Ser  
 645 650 655  
 Asn Asn Pro Asp Ala Asn Leu Thr Leu Asn Thr Asp Ala Gln Glu Lys  
 660 665 670  
 Leu Asn Lys Asn Arg Asp Tyr Tyr Ile Ser Leu Tyr Met Lys Ser Glu  
 675 680 685  
 Lys Asn Thr Gln Cys Glu Ile Thr Ile Asp Gly Glu Ile Tyr Pro Ile  
 690 695 700  
 Thr Thr Lys Thr Val Asn Val Asn Lys Asp Asn Tyr Lys Arg Leu Asp  
 705 710 715 720  
 Ile Ile Ala His Asn Ile Lys Ser Asn Pro Ile Ser Ser Leu His Ile  
 725 730 735  
 Lys Thr Asn Asp Glu Ile Thr Leu Phe Trp Asp Asp Ile Ser Ile Thr  
 740 745 750  
 Asp Val Ala Ser Ile Lys Pro Glu Asn Leu Thr Asp Ser Glu Ile Lys  
 755 760 765  
 Gln Ile Tyr Ser Arg Tyr Gly Ile Lys Leu Glu Asp Gly Ile Leu Ile  
 770 775 780

Asp Lys Lys Gly Gly Ile His Tyr Gly Glu Phe Ile Asn Glu Ala Ser  
 785 790 795 800  
 Phe Asn Ile Glu Pro Leu Pro Asn Tyr Val Thr Lys Tyr Glu Val Thr  
 805 810 815  
 Tyr Ser Ser Glu Leu Gly Pro Asn Val Ser Asp Thr Leu Glu Ser Asp  
 820 825 830  
 Lys Ile Tyr Lys Asp Gly Thr Ile Lys Phe Asp Phe Thr Lys Tyr Ser  
 835 840 845  
 Lys Asn Glu Gln Gly Leu Phe Tyr Asp Ser Gly Leu Asn Trp Asp Phe  
 850 855 860  
 Lys Ile Asn Ala Ile Thr Tyr Asp Gly Lys Glu Met Asn Val Phe His  
 865 870 875 880  
 Arg Tyr Asn Lys

## (2) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2004 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Bacillus cereus*
- (B) STRAIN: AB78
- (C) INDIVIDUAL ISOLATE: NRRL B-21058

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..2001
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /product= "80 kDa protein VIP-1"  
/evidence= EXPERIMENTAL

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATG AAA AGG GAA ATT GAT GAA GAC ACG GAT ACG GAT GGG GAC TCT ATT	48
Met Lys Arg Glu Ile Asp Glu Asp Thr Asp Thr Asp Gly Asp Ser Ile	
1 5 10 15	
CCT GAC CTT TGG GAA GAA AAT GGG TAT ACG ATT CAM AAT AGA ATC GCT	96
Pro Asp Leu Trp Glu Glu Asn Gly Tyr Thr Ile Xaa Asn Arg Ile Ala	
20 25 30	
GTA AAG TGG GAC GAT TCT CTA GCA AGT AAA GGG TAT ACG AAA TTT GTT	144



Val	Lys	Trp	Asp	Asp	Ser	Leu	Ala	Ser	Lys	Gly	Tyr	Thr	Lys	Phe	Val	
	35						40					45				
TCA	AAT	CCA	CTA	GAA	AGT	CAC	ACA	GTT	GGT	GAT	CCT	TAT	ACA	GAT	TAT	192
Ser	Asn	Pro	Leu	Glu	Ser	His	Thr	Val	Gly	Asp	Pro	Tyr	Thr	Asp	Tyr	
	50					55					60					
GAA	AAG	GCA	GCA	AGA	GAT	CTA	GAT	TTG	TCA	AAT	GCA	AAG	GAA	ACG	TTT	240
Glu	Lys	Ala	Ala	Arg	Asp	Leu	Asp	Leu	Ser	Asn	Ala	Lys	Glu	Thr	Phe	
	65				70					75					80	
AAC	CCA	TTG	GTA	GCT	GCT	TTT	CCA	AGT	GTG	AAT	GTT	AGT	ATG	GAA	AAG	288
Asn	Pro	Leu	Val	Ala	Ala	Phe	Pro	Ser	Val	Asn	Val	Ser	Met	Glu	Lys	
				85					90					95		
GTG	ATA	TTA	TCA	CCA	AAT	GAA	AAT	TTA	TCC	AAT	AGT	GTA	GAG	TCT	CAT	336
Val	Ile	Leu	Ser	Pro	Asn	Glu	Asn	Leu	Ser	Asn	Ser	Val	Glu	Ser	His	
			100					105					110			
TCA	TCC	ACG	AAT	TGG	TCT	TAT	ACA	AAT	ACA	GAA	GGT	GCT	TCT	GTT	GAA	384
Ser	Ser	Thr	Asn	Trp	Ser	Tyr	Thr	Asn	Thr	Glu	Gly	Ala	Ser	Val	Glu	
		115					120					125				
GCG	GGG	ATT	GGA	CCA	AAA	GGT	ATT	TCG	TTC	GGA	GTT	AGC	GTA	AAC	TAT	432
Ala	Gly	Ile	Gly	Pro	Lys	Gly	Ile	Ser	Phe	Gly	Val	Ser	Val	Asn	Tyr	
	130					135					140					
CAA	CAC	TCT	GAA	ACA	GTT	GCA	CAA	GAA	TGG	GGA	ACA	TCT	ACA	GGA	AAT	480
Gln	His	Ser	Glu	Thr	Val	Ala	Gln	Glu	Trp	Gly	Thr	Ser	Thr	Gly	Asn	
	145				150					155					160	
ACT	TCG	CAA	TTC	AAT	ACG	GCT	TCA	GCG	GGA	TAT	TTA	AAT	GCA	AAT	GTT	528
Thr	Ser	Gln	Phe	Asn	Thr	Ala	Ser	Ala	Gly	Tyr	Leu	Asn	Ala	Asn	Val	
				165					170					175		
CGA	TAT	AAC	AAT	GTA	GGA	ACT	GGT	GCC	ATC	TAC	GAT	GTA	AAA	CCT	ACA	576
Arg	Tyr	Asn	Asn	Val	Gly	Thr	Gly	Ala	Ile	Tyr	Asp	Val	Lys	Pro	Thr	
			180					185					190			
ACA	AGT	TTT	GTA	TTA	AAT	AAC	GAT	ACT	ATC	GCA	ACT	ATT	ACG	GCG	AAA	624
Thr	Ser	Phe	Val	Leu	Asn	Asn	Asp	Thr	Ile	Ala	Thr	Ile	Thr	Ala	Lys	
		195					200					205				
TCT	AAT	TCT	ACA	GCC	TTA	AAT	ATA	TCT	CCT	GGA	GAA	AGT	TAC	CCG	AAA	672
Ser	Asn	Ser	Thr	Ala	Leu	Asn	Ile	Ser	Pro	Gly	Glu	Ser	Tyr	Pro	Lys	
	210					215					220					
AAA	GGA	CAA	AAT	GGA	ATC	GCA	ATA	ACA	TCA	ATG	GAT	GAT	TTT	AAT	TCC	720
Lys	Gly	Gln	Asn	Gly	Ile	Ala	Ile	Thr	Ser	Met	Asp	Asp	Phe	Asn	Ser	
	225				230					235					240	
CAT	CCG	ATT	ACA	TTA	AAT	AAA	AAA	CAA	GTA	GAT	AAT	CTG	CTA	AAT	AAT	768
His	Pro	Ile	Thr	Leu	Asn	Lys	Lys	Gln	Val	Asp	Asn	Leu	Leu	Asn	Asn	
				245					250					255		
AAA	CCT	ATG	ATG	TTG	GAA	ACA	AAC	CAA	ACA	GAT	GGT	GTT	TAT	AAG	ATA	816
Lys	Pro	Met	Leu	Glu	Thr	Asn	Gln	Thr	Asp	Gly	Val	Tyr	Lys	Ile		
		260					265					270				
AAA	GAT	ACA	CAT	GGA	AAT	ATA	GTA	ACT	GGC	GGA	GAA	TGG	AAT	GGT	GTC	864

Lys Asp Thr His Gly Asn Ile Val Thr Gly Gly Glu Trp Asn Gly Val	
275 280 285	
ATA CAA CAA ATC AAG GCT AAA ACA GCG TCT ATT ATT GTG GAT GAT GGG	912
Ile Gln Gln Ile Lys Ala Lys Thr Ala Ser Ile Ile Val Asp Asp Gly	
290 295 300	
GAA CGT GTA GCA GAA AAA CGT GTA GCG GCA AAA GAT TAT GAA AAT CCA	960
Glu Arg Val Ala Glu Lys Arg Val Ala Ala Lys Asp Tyr Glu Asn Pro	
305 310 315 320	
GAA GAT AAA ACA CCG TCT TTA ACT TTA AAA GAT GCC CTG AAG CTT TCA	1008
Glu Asp Lys Thr Pro Ser Leu Thr Leu Lys Asp Ala Leu Lys Leu Ser	
325 330 335	
TAT CCA GAT GAA ATA AAA GAA ATA GAG GGA TTA TTA TAT TAT AAA AAC	1056
Tyr Pro Asp Glu Ile Lys Glu Ile Glu Gly Leu Leu Tyr Tyr Lys Asn	
340 345 350	
AAA CCG ATA TAC GAA TCG AGC GTT ATG ACT TAC TTA GAT GAA AAT ACA	1104
Lys Pro Ile Tyr Glu Ser Ser Val Met Thr Tyr Leu Asp Glu Asn Thr	
355 360 365	
GCA AAA GAA GTG ACC AAA CAA TTA AAT GAT ACC ACT GGG AAA TTT AAA	1152
Ala Lys Glu Val Thr Lys Gln Leu Asn Asp Thr Thr Gly Lys Phe Lys	
370 375 380	
GAT GTA AGT CAT TTA TAT GAT GTA AAA CTG ACT CCA AAA ATG AAT GTT	1200
Asp Val Ser His Leu Tyr Asp Val Lys Leu Thr Pro Lys Met Asn Val	
385 390 395 400	
ACA ATC AAA TTG TCT ATA CTT TAT GAT AAT GCT GAG TCT AAT GAT AAC	1248
Thr Ile Lys Leu Ser Ile Leu Tyr Asp Asn Ala Glu Ser Asn Asp Asn	
405 410 415	
TCA ATT GGT AAA TGG ACA AAC ACA AAT ATT GTT TCA GGT GGA AAT AAC	1296
Ser Ile Gly Lys Trp Thr Asn Thr Asn Ile Val Ser Gly Gly Asn Asn	
420 425 430	
GGA AAA AAA CAA TAT TCT TCT AAT AAT CCG GAT GCT AAT TTG ACA TTA	1344
Gly Lys Lys Gln Tyr Ser Ser Asn Asn Pro Asp Ala Asn Leu Thr Leu	
435 440 445	
AAT ACA GAT GCT CAA GAA AAA TTA AAT AAA AAT CGT GAC TAT TAT ATA	1392
Asn Thr Asp Ala Gln Glu Lys Leu Asn Lys Asn Arg Asp Tyr Tyr Ile	
450 455 460	
AGT TTA TAT ATG AAG TCA GAA AAA AAC ACA CAA TGT GAG ATT ACT ATA	1440
Ser Leu Tyr Met Lys Ser Glu Lys Asn Thr Gln Cys Glu Ile Thr Ile	
465 470 475 480	
GAT GGG GAG ATT TAT CCG ATC ACT ACA AAA ACA GTG AAT GTG AAT AAA	1488
Asp Gly Glu Ile Tyr Pro Ile Thr Thr Lys Thr Val Asn Val Asn Lys	
485 490 495	
GAC AAT TAC AAA AGA TTA GAT ATT ATA GCT CAT AAT ATA AAA AGT AAT	1536
Asp Asn Tyr Lys Arg Leu Asp Ile Ile Ala His Asn Ile Lys Ser Asn	
500 505 510	
CCA ATT TCT TCA CTT CAT ATT AAA ACG AAT GAT GAA ATA ACT TTA TTT	1584

Pro	Ile	Ser	Ser	Leu	His	Ile	Lys	Thr	Asn	Asp	Glu	Ile	Thr	Leu	Phe		
	515						520					525					
TGG	GAT	GAT	ATT	TCT	ATA	ACA	GAT	GTA	GCA	TCA	ATA	AAA	CCG	GAA	AAT	1632	
Trp	Asp	Asp	Ile	Ser	Ile	Thr	Asp	Val	Ala	Ser	Ile	Lys	Pro	Glu	Asn		
	530					535					540						
TTA	ACA	GAT	TCA	GAA	ATT	AAA	CAG	ATT	TAT	AGT	AGG	TAT	GGT	ATT	AAG	1680	
Leu	Thr	Asp	Ser	Glu	Ile	Lys	Gln	Ile	Tyr	Ser	Arg	Tyr	Gly	Ile	Lys		
	545				550				555						560		
TTA	GAA	GAT	GGA	ATC	CTT	ATT	GAT	AAA	AAA	GGT	GGG	ATT	CAT	TAT	GGT	1728	
Leu	Glu	Asp	Gly	Ile	Leu	Ile	Asp	Lys	Lys	Gly	Gly	Ile	His	Tyr	Gly		
				565					570					575			
GAA	TTT	ATT	AAT	GAA	GCT	AGT	TTT	AAT	ATT	GAA	CCA	TTG	CCA	AAT	TAT	1776	
Glu	Phe	Ile	Asn	Glu	Ala	Ser	Phe	Asn	Ile	Glu	Pro	Leu	Pro	Asn	Tyr		
			580					585					590				
GTG	ACC	AAA	TAT	GAA	GTT	ACT	TAT	AGT	AGT	GAG	TTA	GGA	CCA	AAC	GTG	1824	
Val	Thr	Lys	Tyr	Glu	Val	Thr	Tyr	Ser	Ser	Glu	Leu	Gly	Pro	Asn	Val		
		595					600					605					
AGT	GAC	ACA	CTT	GAA	AGT	GAT	AAA	ATT	TAC	AAG	GAT	GGG	ACA	ATT	AAA	1872	
Ser	Asp	Thr	Leu	Glu	Ser	Asp	Lys	Ile	Tyr	Lys	Asp	Gly	Thr	Ile	Lys		
	610					615					620						
TTT	GAT	TTT	ACC	AAA	TAT	AGT	AAA	AAT	GAA	CAA	GGA	TTA	TTT	TAT	GAC	1920	
Phe	Asp	Phe	Thr	Lys	Tyr	Ser	Lys	Asn	Glu	Gln	Gly	Leu	Phe	Tyr	Asp		
	625				630				635						640		
AGT	GGA	TTA	AAT	TGG	GAC	TTT	AAA	ATT	AAT	GCT	ATT	ACT	TAT	GAT	GGT	1968	
Ser	Gly	Leu	Asn	Trp	Asp	Phe	Lys	Ile	Asn	Ala	Ile	Thr	Tyr	Asp	Gly		
				645					650					655			
AAA	GAG	ATG	AAT	GTT	TTT	CAT	AGA	TAT	AAT	AAA	TAG					2004	
Lys	Glu	Met	Asn	Val	Phe	His	Arg	Tyr	Asn	Lys							
			660						665								

## (2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 667 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met	Lys	Arg	Glu	Ile	Asp	Glu	Asp	Thr	Asp	Thr	Asp	Gly	Asp	Ser	Ile		
1				5					10					15			
Pro	Asp	Leu	Trp	Glu	Glu	Asn	Gly	Tyr	Thr	Ile	Xaa	Asn	Arg	Ile	Ala		
		20						25					30				
Val	Lys	Trp	Asp	Asp	Ser	Leu	Ala	Ser	Lys	Gly	Tyr	Thr	Lys	Phe	Val		
		35					40					45					

Ser Asn Pro Leu Glu Ser His Thr Val Gly Asp Pro Tyr Thr Asp Tyr  
 50 55 60  
 Glu Lys Ala Ala Arg Asp Leu Asp Leu Ser Asn Ala Lys Glu Thr Phe  
 65 70 75 80  
 Asn Pro Leu Val Ala Ala Phe Pro Ser Val Asn Val Ser Met Glu Lys  
 85 90 95  
 Val Ile Leu Ser Pro Asn Glu Asn Leu Ser Asn Ser Val Glu Ser His  
 100 105 110  
 Ser Ser Thr Asn Trp Ser Tyr Thr Asn Thr Glu Gly Ala Ser Val Glu  
 115 120 125  
 Ala Gly Ile Gly Pro Lys Gly Ile Ser Phe Gly Val Ser Val Asn Tyr  
 130 135 140  
 Gln His Ser Glu Thr Val Ala Gln Glu Trp Gly Thr Ser Thr Gly Asn  
 145 150 155 160  
 Thr Ser Gln Phe Asn Thr Ala Ser Ala Gly Tyr Leu Asn Ala Asn Val  
 165 170 175  
 Arg Tyr Asn Asn Val Gly Thr Gly Ala Ile Tyr Asp Val Lys Pro Thr  
 180 185 190  
 Thr Ser Phe Val Leu Asn Asn Asp Thr Ile Ala Thr Ile Thr Ala Lys  
 195 200 205  
 Ser Asn Ser Thr Ala Leu Asn Ile Ser Pro Gly Glu Ser Tyr Pro Lys  
 210 215 220  
 Lys Gly Gln Asn Gly Ile Ala Ile Thr Ser Met Asp Asp Phe Asn Ser  
 225 230 235 240  
 His Pro Ile Thr Leu Asn Lys Lys Gln Val Asp Asn Leu Leu Asn Asn  
 245 250 255  
 Lys Pro Met Met Leu Glu Thr Asn Gln Thr Asp Gly Val Tyr Lys Ile  
 260 265 270  
 Lys Asp Thr His Gly Asn Ile Val Thr Gly Gly Glu Trp Asn Gly Val  
 275 280 285  
 Ile Gln Gln Ile Lys Ala Lys Thr Ala Ser Ile Ile Val Asp Asp Gly  
 290 295 300  
 Glu Arg Val Ala Glu Lys Arg Val Ala Ala Lys Asp Tyr Glu Asn Pro  
 305 310 315 320  
 Glu Asp Lys Thr Pro Ser Leu Thr Leu Lys Asp Ala Leu Lys Leu Ser  
 325 330 335  
 Tyr Pro Asp Glu Ile Lys Glu Ile Glu Gly Leu Leu Tyr Tyr Lys Asn  
 340 345 350  
 Lys Pro Ile Tyr Glu Ser Ser Val Met Thr Tyr Leu Asp Glu Asn Thr  
 355 360 365

Ala Lys Glu Val Thr Lys Gln Leu Asn Asp Thr Thr Gly Lys Phe Lys  
 370 375 380  
 Asp Val Ser His Leu Tyr Asp Val Lys Leu Thr Pro Lys Met Asn Val  
 385 390 395 400  
 Thr Ile Lys Leu Ser Ile Leu Tyr Asp Asn Ala Glu Ser Asn Asp Asn  
 405 410 415  
 Ser Ile Gly Lys Trp Thr Asn Thr Asn Ile Val Ser Gly Gly Asn Asn  
 420 425 430  
 Gly Lys Lys Gln Tyr Ser Ser Asn Asn Pro Asp Ala Asn Leu Thr Leu  
 435 440 445  
 Asn Thr Asp Ala Gln Glu Lys Leu Asn Lys Asn Arg Asp Tyr Tyr Ile  
 450 455 460  
 Ser Leu Tyr Met Lys Ser Glu Lys Asn Thr Gln Cys Glu Ile Thr Ile  
 465 470 475 480  
 Asp Gly Glu Ile Tyr Pro Ile Thr Thr Lys Thr Val Asn Val Asn Lys  
 485 490 495  
 Asp Asn Tyr Lys Arg Leu Asp Ile Ile Ala His Asn Ile Lys Ser Asn  
 500 505 510  
 Pro Ile Ser Ser Leu His Ile Lys Thr Asn Asp Glu Ile Thr Leu Phe  
 515 520 525  
 Trp Asp Asp Ile Ser Ile Thr Asp Val Ala Ser Ile Lys Pro Glu Asn  
 530 535 540  
 Leu Thr Asp Ser Glu Ile Lys Gln Ile Tyr Ser Arg Tyr Gly Ile Lys  
 545 550 555 560  
 Leu Glu Asp Gly Ile Leu Ile Asp Lys Lys Gly Gly Ile His Tyr Gly  
 565 570 575  
 Glu Phe Ile Asn Glu Ala Ser Phe Asn Ile Glu Pro Leu Pro Asn Tyr  
 580 585 590  
 Val Thr Lys Tyr Glu Val Thr Tyr Ser Ser Glu Leu Gly Pro Asn Val  
 595 600 605  
 Ser Asp Thr Leu Glu Ser Asp Lys Ile Tyr Lys Asp Gly Thr Ile Lys  
 610 615 620  
 Phe Asp Phe Thr Lys Tyr Ser Lys Asn Glu Gln Gly Leu Phe Tyr Asp  
 625 630 635 640  
 Ser Gly Leu Asn Trp Asp Phe Lys Ile Asn Ala Ile Thr Tyr Asp Gly  
 645 650 655  
 Lys Glu Met Asn Val Phe His Arg Tyr Asn Lys  
 660 665

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Bacillus cereus*  
(B) STRAIN: AB78  
(C) INDIVIDUAL ISOLATE: NRRL B-21058

**(ix) FEATURE:**

(A) NAME/KEY: Peptide  
(B) LOCATION: 1..16  
(D) OTHER INFORMATION: /note= "N-terminal sequence of protein purified from strain AB78"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Lys Arg Glu Ile Asp Glu Asp Thr Asp Thr Asx Gly Asp Ser Ile Pro  
1 5 10 15

**(2) INFORMATION FOR SEQ ID NO: 9:**

**(1) SEQUENCE CHARACTERISTICS:**

(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

**(ix) FEATURE:**

(A) NAME/KEY: misc feature  
(B) LOCATION: 1..21  
(D) OTHER INFORMATION: /note= "Oligonucleotide probe based on amino acids 3 to 9 of SEQ ID NO:8, using codon usage of *Bacillus thuringiensis*"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GAAATTGATC AAGATACNGA T

**21**

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bacillus thuringiensis  
(B) STRAIN: AB88

(ix) **FEATURE:**

(A) NAME/KEY: Peptide

(B) LOCATION: 1..14

(D) OTHER INFORMATION: /note= "N-terminal amino acid sequence of protein known as anion exchange fraction 23 (smaller)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Xaa Glu Pro Phe Val Ser Ala Xaa Xaa Xaa Gln Xaa Xaa Xaa  
1                  5                  10

**(2) INFORMATION FOR SEQ ID NO: 11:**

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single.

(D) **TOPOLOGY:** linear

(ii) MOLECULE TYPE: peptide

(iii) **HYPOTHETICAL: NO**

(v) **FRAGMENT TYPE:** N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Bacillus thuringiensis*

**(B) STRAIN: AB88**

**(ix) FEATURE:**

(A) NAME/KEY: Peptide

(B) LOCATION: 1..13

(D) OTHER INFORMATION: /note= "N-terminal amino acid sequence of protein known as anion exchange fraction 23 (larger)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Xaa Glu Tyr Glu Asn Val Glu Pro Phe Val Ser Ala Xaa  
1 5 10

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 14 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:  
    (A) ORGANISM: *Bacillus thuringiensis*  
    (B) STRAIN: AB88
- (ix) FEATURE:  
    (A) NAME/KEY: Peptide  
    (B) LOCATION: 1..14  
    (D) OTHER INFORMATION: /note= "N-terminal sequence of 80  
        kDa VIP active against *Agrotis ipsilon*"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
- |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Asn | Lys | Asn | Asn | Thr | Lys | Leu | Pro | Thr | Arg | Ala | Leu | Pro |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     |
- (2) INFORMATION FOR SEQ ID NO: 13:
- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 15 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:  
    (A) ORGANISM: *Bacillus thuringiensis*  
    (B) STRAIN: AB88
- (ix) FEATURE:  
    (A) NAME/KEY: Peptide  
    (B) LOCATION: 1..15  
    (D) OTHER INFORMATION: /note= "N-terminal amino acid  
        sequence of 35 kDa VIP active against *Agrotis ipsilon*"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
- |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Leu | Ser | Glu | Asn | Thr | Gly | Lys | Asp | Gly | Gly | Tyr | Ile | Val | Pro |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |
- (2) INFORMATION FOR SEQ ID NO: 14:



- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 9 amino acids
    - (B) TYPE: amino acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: peptide
  - (iii) HYPOTHETICAL: NO
  - (v) FRAGMENT TYPE: N-terminal
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: *Bacillus thuringiensis*
  - (ix) FEATURE:
    - (A) NAME/KEY: Peptide
    - (B) LOCATION: 1..9
    - (D) OTHER INFORMATION: /note= "N-terminal sequence of a 130 kDa delta-endotoxin"
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:  
Met Asp Asn Asn Pro Asn Ile Asn Glu  
1 5
- (2) INFORMATION FOR SEQ ID NO: 15:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 9 amino acids
    - (B) TYPE: amino acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: peptide
  - (iii) HYPOTHETICAL: NO
  - (v) FRAGMENT TYPE: N-terminal
  - (ix) FEATURE:
    - (A) NAME/KEY: Peptide
    - (B) LOCATION: 1..9
    - (D) OTHER INFORMATION: /note= "N-terminal sequence of 80 kDa delta-endotoxin"
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:  
Met Asp Asn Asn Pro Asn Ile Asn Glu  
1 5
- (2) INFORMATION FOR SEQ ID NO: 16:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 11 amino acids
    - (B) TYPE: amino acid

(C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: *Bacillus thuringiensis*

(ix) FEATURE:  
 (A) NAME/KEY: Peptide  
 (B) LOCATION: 1..11  
 (D) OTHER INFORMATION: /note= "N-terminal sequence from 60 kDa delta-endotoxin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Met Asn Val Leu Asn Ser Gly Arg Thr Thr Ile  
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2655 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

ATGAAGAACA TGAAGAAGAA GCTGGCCAGC GTGGTGACCT GCACCCTGCT GGCCCCCATG	60
TTCCTGAACG GCAACGTGAA CGCCGTGTAC GCCGACAGCA AGACCAACCA GATCAGCACC	120
ACCCAGAAGA ACCAGCAGAA GGAGATGGAC CGCAAGGGCC TGCTGGGCTA CTACTTCAAG	180
GGCAAGGACT TCAGCAACCT GACCATGTTT GCCCCACGC GTGACAGCAC CCTGATCTAC	240
GACCAGCAGA CCGCCAACAA GCTGCTGGAC AAGAAGCAGC AGGAGTACCA GAGCATCCGC	300
TGGATCGGCC TGATCCAGAG CAAGGAGACC GGCGACTTCA CCTTCAACCT GAGCGAGGAC	360
GAGCAGGCCA TCATCGAGAT CAACGGCAAG ATCATCAGCA ACAAGGGCAA GGAGAAGCAG	420
GTGGTGACCC TGGAGAAGGG CAAGCTGGTG CCCATCAAGA TCGAGTACCA GAGCGACACC	480
AAGTTCAACA TCGACAGCAA GACCTTCAAG GAGCTGAAGC TTTTCAAGAT CGACAGCCAG	540

AACCAGCCCC AGCAGGTGCA GCAGGACGAG CTGCGCAACC CCGAGTTCAA CAAGAAGGAG	600
AGCCAGGAGT TCCTGGCCAA GCCCAGCAAG ATCAACCTGT TCACCCAGCA GATGAAGCGC	660
GAGATCGACG AGGACACCGA CACCGACGGC GACAGCATCC CCGACCTGTG GGAGGAGAAC	720
GGCTACACCA TCCAGAACCG CATCGCCGTG AAGTGGGACG ACAGCCTGGC TAGCAAGGGC	780
TACACCAAGT TCGTGAGCAA CCCCCTGGAG AGCCACACCG TGGGCGACCC CTACACCGAC	840
TACGAGAAGG CCGCCCGCGA CCTGGACCTG AGCAACGCCA AGGAGACCTT CAACCCCCTG	900
GTGGCCGCCT TCCCCAGCGT GAACGTGAGC ATGGAGAAGG TGATCCTGAG CCCCACGAG	960
AACCTGAGCA ACAGCGTGGA GAGCCACTCG AGCACCAACT GGAGCTACAC CAACACCGAG	1020
GGCGCCAGCG TGGAGGCCGG CATCGGTCCC AAGGGCATCA GCTTCGGCGT GAGCGTGAAC	1080
TACCAGCACA GCGAGACCGT GGCCCAGGAG TGGGGCACCA GCACCGGCAA CACCAGCCAG	1140
TTCAACACCG CCAGCGCCGG CTACCTGAAC GCCAACGTGC GCTACAACAA CGTGGGCACC	1200
GGCGCCATCT ACGACGTGAA GCCCACCACC AGCTTCGTGC TGAACAACGA CACCATCGCC	1260
ACCATCACCG CCAAGTCGAA TTCCACCGCC CTGAACATCA GCCCCGCGA GAGCTACCCC	1320
AAGAAGGGCC AGAACGGCAT CGCCATCACC AGCATGGACG ACTTCAACAG CCACCCCATC	1380
ACCTGAACA AGAAGCAGGT GGACAACCTG CTGAACAACA AGCCCATGAT GCTGGAGACC	1440
AACCAGACCG ACGGCGTCTA CAAGATCAAG GACACCCACG GCAACATCGT GACCGGCGGC	1500
GAGTGGAACG GCGTGATCCA GCAGATCAAG GCCAAGACCG CCAGCATCAT CGTCGACGAC	1560
GGCGAGCGCG TGCCCGAGAA GCGCGTGGCC GCCAAGGACT ACGAGAACCC CGAGGACAAG	1620
ACCCCCAGCC TGACCCTGAA GGACGCCCTG AAGCTGAGCT ACCCCGACGA GATCAAGGAG	1680
ATCGAGGGCC TGCTGTACTA CAAGAACAAG CCCATCTACG AGAGCAGCGT GATGACCTAT	1740
CTAGACGAGA ACACCGCCAA GGAGGTGACC AAGCAGCTGA ACGACACCAC CGGCAAGTTC	1800
AAGGACGTGA GCCACCTGTA CGACGTGAAG CTGACCCCCA AGATGAACGT GACCATCAAG	1860
CTGAGCATCC TGTACGACAA CGCCGAGAGC AACGACAACA GCATCGGCAA GTGGACCAAC	1920
ACCAACATCG TGAGCGGCGG CAACAACGGC AAGAAGCAGT ACAGCAGCAA CAACCCCGAC	1980
GCCAACCTGA CCCTGAACAC CGACGCCCAG GAGAAGCTGA ACAAGAACCG CGACTACTAC	2040
ATCAGCCTGT ACATGAAGAG CGAGAAGAAC ACCCAGTGCG AGATCACCAT CGACGGCGAG	2100
ATATACCCCA TCACCACCAA GACCGTGAAC GTGAACAAGG ACAACTACAA GCGCCTGGAC	2160
ATCATCGCCC ACAACATCAA GAGCAACCCC ATCAGCAGCC TGCACATCAA GACCAACGAC	2220
GAGATCACCC TGTTCTGGGA CGACATATCG ATTACCGACG TCGCCAGCAT CAAGCCCGAG	2280
AACCTGACCG ACAGCGAGAT CAAGCAGATA TACAGTCGCT ACGGCATCAA GCTGGAGGAC	2340

GGCATCCTGA TCGACAAGAA GGGCGGCATC CACTACGGCG AGTTCATCAA CGAGGCCAGC	2400
TTCAACATCG AGCCCCTGCA GAACTACGTG ACCAAGTACG AGGTGACCTA CAGCAGCGAG	2460
CTGGGCCCCA ACGTGAGCGA CACCCTGGAG AGCGACAAGA TTTACAAGGA CGGCACCATC	2520
AAGTTCGACT TCACCAAGTA CAGCAAGAAC GAGCAGGGCC TGTCTACGA CAGCGGCCTG	2580
AACTGGGACT TCAAGATCAA CGCCATCACC TACGACGGCA AGGAGATGAA CGTGTCCAC	2640
CGCTACAACA AGTAG	2655

## (2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2010 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

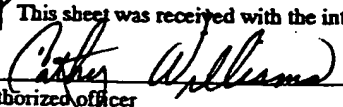
## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GGATCCATGA AGCGCGAGAT CGACGAGGAC ACCGACACCG ACGGCGACAG CATCCCCGAC	60
CTGTGGGAGG AGAACGGCTA CACCATCCAG AACCGCATCG CCGTGAAGTG GGACGACAGC	120
CTGGCTAGCA AGGGCTACAC CAAGTTCGTG AGCAACCCCC TGGAGAGCCA CACCGTGGGC	180
GACCCCTACA CCGACTACGA GAAGGCCGCC CGCGACCTGG ACCTGAGCAA CGCCAAGGAG	240
ACCTTCAACC CCCTGGTGGC CGCCTTCCCC AGCGTGAACG TGAGCATGGA GAAGGTGATC	300
CTGAGCCCCA ACGAGAACCT GAGCAACAGC GTGGAGAGCC ACTCGAGCAC CAACTGGAGC	360
TACACCAACA CCGAGGGCGC CAGCGTGGAG GCCGGCATCG GTCCAAGGG CATCAGCTTC	420
GGCGTGAGCG TGAACCTACCA GCACAGCGAG ACCGTGGCCC AGGAGTGGGG CACCAGCACC	480
GGCAACACCA GCCAGTTCAA CACCGCCAGC GCCGGCTACC TGAACGCCAA CGTGCGCTAC	540
AACAACGTGG GCACCGGCGC CATCTACGAC GTGAAGCCCA CCACCAGCTT CGTGCTGAAC	600
AACGACACCA TCGCCACCAT CACCGCCAAG TCGAATTCCA CCGCCCTGAA CATCAGCCCC	660
GGCGAGAGCT ACCCCAAGAA GGGCCAGAAC GGCATCGCCA TCACCAGCAT GGACGACTTC	720
AACAGCCACC CCATCACCCCT GAACAAGAAG CAGGTGGACA ACCTGCTGAA CAACAAGCCC	780
ATGATGCTGG AGACCAACCA GACCGACGGC GTCTACAAGA TCAAGGACAC CCACGGCAAC	840
ATCGTGACCG GCGGCGAGTG GAACGGCGTG ATCCAGCAGA TCAAGGCCAA GACCGCCAGC	900

ATCATCGTCG ACGACGGCGA GCGCGTGGCC GAGAAGCGCG TGGCCGCCAA GGACTACGAG	960
AACCCCGAGG ACAAGACCCC CAGCCTGACC CTGAAGGACG CCCTGAAGCT GAGCTACCCC	1020
GACGAGATCA AGGAGATCGA GGGCCTGCTG TACTACAAGA ACAAGCCCAT CTACGAGAGC	1080
AGCGTGATGA CCTATCTAGA CGAGAACACC GCCAAGGAGG TGACCAAGCA GCTGAACGAC	1140
ACCACCGGCA AGTTCAAGGA CGTGAGCCAC CTGTACGACG TGAAGCTGAC CCCCAAGATG	1200
AACGTGACCA TCAAGCTGAG CATCCTGTAC GACAACGCCG AGAGCAACGA CAACAGCATC	1260
GGCAAGTGGA CCAACACCAA CATCGTGAGC GGCGGCAACA ACGGCAAGAA GCAGTACAGC	1320
AGCAACAACC CCGACGCCAA CCTGACCCTG AACACCGACG CCCAGGAGAA GCTGAACAAG	1380
AACCGCGACT ACTACATCAG CCGTGTACATG AAGAGCGAGA AGAACACCCA GTGCGAGATC	1440
ACCATCGACG GCGAGATATA CCCCATCACC ACCAAGACCG TGAACGTGAA CAAGGACAAC	1500
TACAAGCGCC TGGACATCAT CGCCCACAAC ATCAAGAGCA ACCCCATCAG CAGCCTGCAC	1560
ATCAAGACCA ACGACGAGAT CACCCTGTTC TGGGACGACA TATCGATTAC CGACGTCGCC	1620
AGCATCAAGC CCGAGAACCT GACCGACAGC GAGATCAAGC AGATATACAG TCGCTACGGC	1680
ATCAAGCTGG AGGACGGCAT CCTGATCGAC AAGAAGGGCG GCATCCACTA CGGCGAGTTC	1740
ATCAACGAGG CCAGCTTCAA CATCGAGCCC CTGCAGAACT ACGTGACCAA GTACGAGGTG	1800
ACCTACAGCA GCGAGCTGGG CCCCAACGTG AGCGACACCC TGGAGAGCGA CAAGATTTAC	1860
AAGGACGGCA CCATCAAGTT CGACTTCACC AAGTACAGCA AGAACGAGCA GGGCCTGTTC	1920
TACGACAGCG GCCTGAAGTG GGACTTCAAG ATCAACGCCA TCACCTACGA CGGCAAGGAG	1980
ATGAACGTGT TCCACCGCTA CAACAAGTAG	2010

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>26</u> , line <u>12-14</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depositary Authority</b>	
Address of depositary institution (including postal code and country) <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>18 March 1993 (18.03.93)</b>	Accession Number <b>NRRL B-21058</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
<b>We request the Expert Solution where available</b>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<b>For receiving Office use only</b> <input checked="" type="checkbox"/> This sheet was received with the international application  Authorized officer	<b>For International Bureau use only</b> <input type="checkbox"/> This sheet was received by the International Bureau on:  Authorized officer

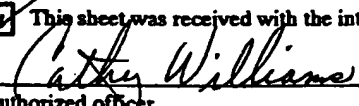
## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>41</u> , line <u>1-3</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depositary Authority</b>	
Address of depositary institution <i>(including postal code and country)</i>  <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>18 March 1993 (18.03.93)</b>	Accession Number <b>NRRL B-21059</b>
<b>C. ADDITIONAL INDICATIONS</b> <i>(leave blank if not applicable)</i> <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
We request the Expert Solution where available	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> <i>(if the indications are not for all designated States)</i>	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> <i>(leave blank if not applicable)</i>	
The indications listed below will be submitted to the International Bureau later <i>(specify the general nature of the indications e.g., "Accession Number of Deposit")</i>	
<div style="border: 1px solid black; padding: 2px;"><div style="text-align: center; font-weight: bold; font-size: small;">For receiving Office use only</div><div style="display: flex; align-items: center;"><input checked="" type="checkbox"/> This sheet was received with the international application</div><div style="margin-top: 10px;"><div style="border-top: 1px solid black; width: 100%; text-align: center; font-size: x-small;">Authorized officer</div></div></div>	<div style="border: 1px solid black; padding: 2px;"><div style="text-align: center; font-weight: bold; font-size: small;">For International Bureau use only</div><div style="display: flex; align-items: center;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div><div style="margin-top: 10px;"><div style="border-top: 1px solid black; width: 100%; text-align: center; font-size: x-small;">Authorized officer</div></div></div>

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

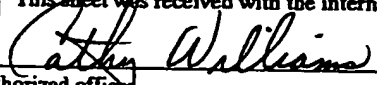
<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>48</u> , line <u>18-20</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depository Authority</b>	
Address of depositary institution (including postal code and country)  <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>18 March 1993 (18.03.93)</b>	Accession Number <b>NRRL B-21060</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>We request the Expert Solution where available</b>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div style="text-align: right; border-bottom: 1px solid black; margin-bottom: 5px;">For receiving Office use only</div> <div style="border: 1px solid black; padding: 5px;"><input checked="" type="checkbox"/> This sheet was received with the international application   Authorized officer</div>	<div style="text-align: right; border-bottom: 1px solid black; margin-bottom: 5px;">For International Bureau use only</div> <div style="border: 1px solid black; padding: 5px;"><input type="checkbox"/> This sheet was received by the International Bureau on:  Authorized officer</div>



## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>41</u> , line <u>1-3</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depositary Authority</b>	
Address of depositary institution <i>(including postal code and country)</i>  <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>18 March 1993 (18.03.93)</b>	Accession Number <b>NRRL B-21061</b>
<b>C. ADDITIONAL INDICATIONS</b> <i>(leave blank if not applicable)</i> <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>We request the Expert Solution where available</b>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> <i>(if the indications are not for all designated States)</i>	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> <i>(leave blank if not applicable)</i>	
The indications listed below will be submitted to the International Bureau later <i>(specify the general nature of the indications e.g., "Accession Number of Deposit")</i>	

<div style="text-align: right; font-weight: bold; font-size: small;">For receiving Office use only</div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;"><input checked="" type="checkbox"/> This sheet was received with the international application <div style="text-align: center; margin-top: 10px;"> Cathy Williams Authorized officer</div></div>	<div style="text-align: right; font-weight: bold; font-size: small;">For International Bureau use only</div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;"><input type="checkbox"/> This sheet was received by the International Bureau on: <div style="text-align: center; margin-top: 10px;">_____ Authorized officer</div></div>
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>42</u> , line <u>8-10</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depository Authority</b>	
Address of depositary institution (including postal code and country)  <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>09 March 1994 (09.03.94)</b>	Accession Number <b>NRRL B-21221</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>We request the Expert Solution where available</b>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div style="border-bottom: 1px solid black; margin-bottom: 5px;"><input checked="" type="checkbox"/> For receiving Office use only</div> <div style="border-bottom: 1px solid black; margin-bottom: 5px;"><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div style="border-bottom: 1px solid black; margin-bottom: 5px;"></div> <div>Authorized officer</div>	<div style="border-bottom: 1px solid black; margin-bottom: 5px;"><input type="checkbox"/> For International Bureau use only</div> <div style="border-bottom: 1px solid black; margin-bottom: 5px;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="border-bottom: 1px solid black; margin-bottom: 5px;"></div> <div>Authorized officer</div>

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A. The indications made below relate to the microorganism referred to in the description</b> on page <u>42</u> , line <u>21-24</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL)</b> <b>International Depositary Authority</b>	
Address of depositary institution (including postal code and country)  <b>1815 N. University Street</b> <b>Peoria, IL 61604</b> <b>USA</b>	
Date of deposit <b>09 March 1994 (09.03.94)</b>	Accession Number <b>NRRL B-21222</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>We request the Expert Solution where available</b>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<div style="text-align: right; font-weight: bold; font-size: small;">For receiving Office use only</div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;"><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;"><b>Cathy Williams</b> Authorized officer</div>	<div style="text-align: right; font-weight: bold; font-size: small;">For International Bureau use only</div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;">Authorized officer</div>
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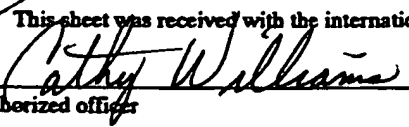
## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>43</u> , line <u>15-18</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depositary Authority</b>	
Address of depositary institution (including postal code and country)  <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>09 March 1994 (09.03.94)</b>	Accession Number <b>NRRL B-21223</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>We request the Expert Solution where available</b>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div style="text-align: right; border-bottom: 1px solid black; margin-bottom: 5px;">For receiving Office use only</div> <div style="display: flex; align-items: center;"><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div style="border-top: 1px solid black; height: 40px; margin-top: 5px;"></div> <div style="border-top: 1px solid black; padding-top: 5px;">Authorized officer</div>	<div style="text-align: right; border-bottom: 1px solid black; margin-bottom: 5px;">For International Bureau use only</div> <div style="display: flex; align-items: center;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="border-top: 1px solid black; height: 40px; margin-top: 5px;"></div> <div style="border-top: 1px solid black; padding-top: 5px;">Authorized officer</div>

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

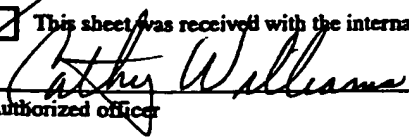
(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>53</u> , line <u>18</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depository Authority</b>	
Address of depositary institution (including postal code and country) <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>09 March 1994 (09.03.94)</b>	Accession Number <b>NRRL B-21224</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>  <b>We request the Expert Solution where available</b>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<b>For receiving Office use only</b> <input checked="" type="checkbox"/> This sheet was received with the international application  Authorized officer	<b>For International Bureau use only</b> <input type="checkbox"/> This sheet was received by the International Bureau on:  Authorized officer

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

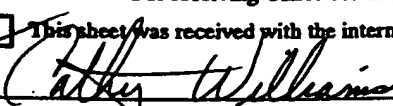
(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>51</u> , line <u>8-10</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depositary Authority</b>	
Address of depositary institution (including postal code and country)  <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit  <b>09 March 1994 (09.03.94)</b>	Accession Number  <b>NRRL B-21225</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
 <b>We request the Expert Solution where available</b>  	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")   	

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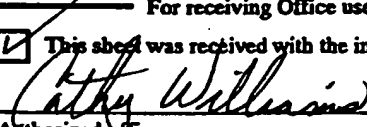
## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>3-6</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depositary Authority</b>	
Address of depositary institution (including postal code and country)  <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>09 March 1994 (09.03.94)</b>	Accession Number <b>NRRL B-21226</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>We request the Expert Solution where available</b>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div style="border: 1px solid black; padding: 5px;"><div style="text-align: center;"><b>For receiving Office use only</b></div><div><input checked="" type="checkbox"/> This sheet was received with the international application</div><div style="text-align: center;"> Authorized officer</div></div>	<div style="border: 1px solid black; padding: 5px;"><div style="text-align: center;"><b>For International Bureau use only</b></div><div><input type="checkbox"/> This sheet was received by the International Bureau on:</div><div style="text-align: center;">Authorized officer</div></div>

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>3-6</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depository Authority</b>	
Address of depositary institution (including postal code and country)  <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>09 March 1994 (09.03.94)</b>	Accession Number <b>NRRL B-21227</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>We request the Expert Solution where available</b>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div style="text-align: right; border-bottom: 1px solid black; margin-bottom: 5px;">For receiving Office use only</div> <div style="display: flex; align-items: center;"><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div style="text-align: center; margin-top: 10px;"> Authorized officer</div>	<div style="text-align: right; border-bottom: 1px solid black; margin-bottom: 5px;">For International Bureau use only</div> <div style="display: flex; align-items: center;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="text-align: center; margin-top: 10px;">Authorized officer</div>



## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>3-6</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depository Authority</b>	
Address of depositary institution (including postal code and country)  <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>09 March 1994 (09.03.94)</b>	Accession Number <b>NRRL B-21228</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>We request the Expert Solution where available</b>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>3-6</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depository Authority</b>	
Address of depositary institution (including postal code and country)  <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>09 March 1994 (09.03.94)</b>	Accession Number <b>NRRL B-21229</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
<b>We request the Expert Solution where available</b>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div style="text-align: right; border-bottom: 1px solid black; margin-bottom: 5px;">For receiving Office use only</div> <div style="display: flex; justify-content: space-between; align-items: flex-start;"><div style="width: 40%;"><input checked="" type="checkbox"/> This sheet was received with the international application</div><div style="width: 60%; text-align: center;"> Authorized officer</div></div>	<div style="text-align: right; border-bottom: 1px solid black; margin-bottom: 5px;">For International Bureau use only</div> <div style="display: flex; justify-content: space-between; align-items: flex-start;"><div style="width: 40%;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div><div style="width: 60%; text-align: center;"> Authorized officer</div></div>

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>53</u> , line <u>1-4</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <b>Agricultural Research Culture Collection (NRRL) International Depositary Authority</b>	
Address of depositary institution (including postal code and country) <b>1815 N. University Street Peoria, IL 61604 USA</b>	
Date of deposit <b>09 March 1994 (09.03.94)</b>	Accession Number <b>NRRL B-21230</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>  <b>We request the Expert Solution where available</b>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")    	
<b>For receiving Office use only</b> <input checked="" type="checkbox"/> This sheet was received with the international application <i>Cathy Williams</i> Authorized officer	<b>For International Bureau use only</b> <input type="checkbox"/> This sheet was received by the International Bureau on:  Authorized officer

What is claimed is:

1. A substantially purified Bacillus strain which produces a pesticidal protein during vegetative growth.
- 5 2. The Bacillus strain of claim 1 wherein said Bacillus is selected from a Bacillus species listed in Table 11.
3. The Bacillus strain of claim 1 wherein said protein is capable of killing pests selected from insects, fungi, bacteria, nematodes, mites, ticks, protozoan pathogens, animal parasites, and the like.
- 10 4. The Bacillus strain of claim 3, wherein said protein is capable of killing insects selected from orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Mallophaga, Anoplura, Siphonaptera, or Trichoptera.
5. The Bacillus strain of claim 4, wherein said coleopteran species is a Diabrotica.
- 15 6. The Bacillus strain of claim 5, wherein said Diabrotica is Diabrotica virgifera virgifera or Diabrotica longicornis barberi.
7. The Bacillus strain of claim 4, wherein said lepidopteran species is an Agrotis.
8. The Bacillus strain of claim 7, wherein said Agrotis is Agrotis ipsilon.
9. The Bacillus strain of claim 2, wherein said Bacillus is Bacillus cereus.
- 20 10. The Bacillus strain of claim 9, wherein said Bacillus cereus is Bacillus cereus having Accession No. NRRL B-21058.
11. The Bacillus strain of claim 2, wherein said Bacillus is Bacillus thuringensis.
12. The Bacillus strain of claim 11, wherein said Bacillus thuringensis is Bacillus thuringensis having Accession No. NRRL B-21060.

13. The *Bacillus* strain of claim 2, wherein said protein has a molecular weight of 30 kDa or greater.
14. The *Bacillus* strain of claim 13, wherein said protein has a molecular weight of about 60 to about 100 kDa.
- 5 15. The *Bacillus* strain of claim 14, wherein said protein has a molecular weight of about 80 kDa.
16. The *Bacillus* strain of claim 15, wherein said protein has the sequence given in SEQ. ID. NO:7.
17. The *Bacillus* strain of claim 14, wherein said protein has a molecular weight of about 100  
10 kDa.
18. The *Bacillus* strain of claim 17, wherein said protein has the sequence given in SEQ. ID. NO:5.
19. A substantially pure pesticidal protein isolatable during the vegetative growth phase of *Bacillus* spp. or analogs and active fragments thereof.
- 15 20. The pesticidal protein of claim 19 wherein said *Bacillus* is selected from a *Bacillus* species listed in Table 11.
21. The pesticidal protein of claim 20, wherein said insects are selected from orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Mallophaga, Anoplura, Siphonaptera, or  
20 Trichoptera.
22. The pesticidal protein of claim 21, wherein said coleopteran species is a *Diabrotica*.
23. The pesticidal protein of claim 22, wherein said *Diabrotica* is *Diabrotica virgifera virgifera* or *Diabrotica longicornis barberi*.
24. The pesticidal protein of claim 21, wherein said lepidopteran species is an *Agrotis*.
- 25 25. The pesticidal protein of claim 24, wherein said *Agrotis* is *Agrotis ipsilon*.

26. The pesticidal protein of claim 19, wherein said Bacillus is Bacillus cereus.
27. The pesticidal protein of claim 26, wherein said Bacillus cereus is Bacillus cereus having Accession No. B-21058.
28. The pesticidal protein of claim 19, wherein said Bacillus is Bacillus thuringensis.
- 5 29. The pesticidal protein of claim 28, wherein said Bacillus thuringensis is Bacillus thuringensis selected from Accession Numbers NRRL B-21060, NRRL B-21224, NRRL B-21225, NRRL B-21226 and NRRL B-21227.
30. The pesticidal protein of claim 19, wherein said protein has a molecular weight of 30 kDa or greater.
- 10 31. The pesticidal protein of claim 30, wherein said protein has a molecular weight of about 60 to about 100 kDa.
32. The pesticidal protein of claim 31, wherein said protein has a molecular weight of about 80 kDa.
33. The pesticidal protein of claim 32, wherein said protein has the sequence given in SEQ  
15 ID NO:7.
34. The pesticidal protein of claim 31, wherein said protein has the sequence given in SEQ ID NO:5.
35. The pesticidal protein of claim 19, wherein said protein comprises an N-terminal sequence as set forth in SEQ ID NOS:10 or 11.
- 20 36. A substantially pure nucleotide sequence which encodes the protein of claim 19.
37. A substantially pure nucleotide sequence which encodes the protein of claim 33.
38. A substantially pure nucleotide sequence which encodes the protein of claim 34.
39. A substantially pure nucleotide sequence which encodes the protein of claim 35.
40. The nucleotide sequence of claim 36, wherein said sequence has been optimized for  
25 expression in a plant.

41. The nucleotide sequence of claim 40, wherein said plant is selected from maize, soybean, cotton, wheat, sunflower, tomato, potato, and oilseed rape.
42. The nucleotide sequence of claim 37, wherein said sequence has been optimized for expression in a plant.
- 5 43. The nucleotide sequence of claim 42, wherein said plant is selected from maize, soybean, cotton, wheat, sunflower, tomato, potato, and oilseed rape.
44. The nucleotide sequence of claim 38, wherein said sequence has been optimized for expression in a plant.
45. The nucleotide sequence of claim 44, wherein said sequence is set forth in SEQ ID NO:  
10 18.
46. The nucleotide sequence of claim 39, wherein said sequence has been optimized for expression in a plant.
47. The nucleotide sequence of claim 46, wherein said sequence is set forth in SEQ ID NO:  
17.
- 15 48. The nucleotide sequence of claim 36, wherein said sequence has been optimized for expression in a microorganism.
49. The nucleotide sequence of claim 48, wherein said microorganism is selected from Bacillus, Pseudomonas, Saccharomyces, Clavibacter, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Agrobacterium, insect pathogenic viruses, fungi,  
20 protozoans and nematodes.
50. A plant which has been stably transformed with the nucleotide sequence of any one of claims 36-47
51. The plant of claim 48, wherein said plant is a maize plant.
52. The nucleotide sequence of claim 36, wherein said sequence is essentially the sequence  
25 of E. coli clone P5-4 having Accession No. NRRL B-21059.

53. The nucleotide sequence of claim 36, wherein said sequence is essentially the sequence of E. coli clone P3-12 having Accession No. NRRL B-21061.
54. The nucleotide sequence of claim 36, wherein said sequence is contained in E. coli clone pCIB 6022 having Accession No. NRRL B-21222.
- 5 55. The nucleotide sequence of claim 54 wherein said sequence is given as VIP-1 in SEQ ID NO:1.
56. An auxiliary protein which enhances the pesticidal activity of a pesticidal protein.
57. The auxiliary protein of claim 56 wherein said pesticidal protein is from Bacillus.
58. The auxiliary protein of claim 57 wherein said pesticidal protein is from B. cereus.
- 10 59. The auxiliary protein of claim 58 wherein said pesticidal protein is from strain AB78.
60. The auxiliary protein of claim 56 wherein said auxiliary protein is from Bacillus.
61. The auxiliary protein of claim 60 wherein said auxiliary protein is from B. cereus.
62. The auxiliary protein of claim 61 wherein said auxiliary protein is from strain AB78.
63. A substantially pure nucleotide sequence which encodes the auxiliary protein of any one of claims 56, 60, 61, and 62.
- 15 64. The nucleotide sequence of claim 63 wherein said sequence is contained in E. coli clone pCIB6022 having Accession No. NRRL B-21222.
65. The Bacillus strain of claim 1 wherein said strain is AB88 having Accession No. NRRL B-21225.
- 20 66. The Bacillus strain of claim 1 wherein said strain is AB289 having Accession No. NRRL B-21227.
67. The Bacillus strain of claim 1 wherein said strain is AB294 having Accession No. NRRL B-21229.
68. The Bacillus strain of claim 1 wherein said strain is AB359 having Accession No. NRRL B-21226.
- 25



69. The Bacillus strain of claim 1 wherein said strain is AB59 having Accession No. NRRL B-21228.
70. The Bacillus strain of claim 1 wherein said strain is AB256 having Accession No. NRRL B-21230.

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 94/03131

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/31 C12N15/32 C12N15/82 A01H5/00 A01N63/00  
C12P1/04 C12N1/21 //C12Q1/68, C12P21/08, (C12P1/04,  
C12R1:07), (C12N1/21, C12R1:07, 1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N C12P A01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO, A, 91 16434 (ECOGEN, INC.) 31 October 1991</p> <p>see page 11, line 31 - page 13, line 15 see page 23, line 20 - page 24, line 3 see page 28, line 1 - page 29, line 25 see examples 9-11 see figure 2</p> <p style="text-align: center;">--- -/--</p>	<p>1-4, 11, 13-15, 19-21, 28, 30-32, 35, 36, 39-41, 46, 48-50</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

21 June 1994

Date of mailing of the international search report

22.07.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Andres, S

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 94/03131

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,88 08880 (ECOGEN, INC.) 17 November 1988  see page 7, line 5 - page 8, line 9 see page 18, line 21 - page 20, line 10 see page 41 - page 42 ---	1-4, 9, 13, 14, 19-21, 26, 30, 31, 36, 48-50
X	CURR MICROBIOL 17 (6). 1988. 347-350 SEKAR, V. 'THE INSECTICIDAL CRYSTAL PROTEIN GENE IS EXPRESSED IN VEGETATIVE CELLS OF BACILLUS -THURINGIENSIS-VAR- TENEBRIONIS.'	1-4, 11, 13, 14, 19-21, 28, 30, 31, 36
Y	see the whole document ---	5, 6, 40, 41
Y	BIOTECHNOLOGY vol. 11, February 1993, NEW YORK US pages 194 - 200 KOZIEL, M. ET AL. 'Field performance of elite transgenic maize plants expressing an insecticidal protein derived from Bacillus thuringiensis' see the whole document ---	40, 41
X	WO,A,90 13651 (IMPERIAL CHEMICAL INDUSTRIES PLC) 15 November 1990  see page 4, line 17 - line 30 see page 5, line 36 - page 7, line 17 see examples 9-21 ---	19-23, 28, 30-32, 35, 36, 39-41, 51
Y		5, 6
X	BIOSCIENCE, BIOTECHNOLOGY, AND BIOCHEMISTRY vol. 56, no. 9, September 1992 pages 1429 - 1433 YOSHISUE, H. ET AL. 'Effects of Bacillus thuringiensis var. israelensis 20-kDa protein on production of the Bti 130-kDa crystal protein in Escherichia coli' see abstract ---	56, 57, 60, 63
X	PLASMID vol. 16, no. 3, November 1986 page 230 SHIVAKUMAR, A. ET AL. 'Cloned crystal protein genes express vegetatively in Bacillus subtilis' see abstract ---	1, 2
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-/--

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 94/03131

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO,A,91 16432 (PLANT GENETIC SYSTEMS, N.V.) 31 October 1991 cited in the application see page 6, line 14 - page 7 see examples see claims</p> <p style="text-align: center;">---</p>	40,41
A	<p>MICROBIOLOGICAL REVIEWS vol. 53, no. 2 , June 1989 , WASHINGTON DC, US pages 242 - 255 HÖFTE, H. &amp; WHITELEY, H. 'Insecticidal crystal proteins of Bacillus thuringiensis'</p> <p style="text-align: center;">-----</p>	

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/03131

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
**Claim 51 relating to an engineered plant refers to claim 48 relating to an engineered microorganism. Therefore, claim 51 has been searched independently of claim 48.**
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 94/03131

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